National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC)

Protocol

Originally Approved:
July 12, 2007 v1

Updated:
June 9, 2008 v1.1

December 13, 2010

Version 2

Supported under NHLBI contract HHSN268201000048C/N01-HV-08238 with RTI International

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Abstract

The purpose of the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC) is to improve the diagnosis and management of patients receiving treatment for thoracic aortic aneurysms and dissections, which are known or suspected to be genetically induced, through the creation of a data and specimen repository. Data to be collected include medical, surgical, and lifestyle, as well as blood and tissue samples. RTI International will serve as the Data Coordinating Center (DCC) and prime contractor. The Registry was designed and patient enrollment will be accomplished through the collaboration of eight GenTAC clinical centers (GCCs): Baylor College of Medicine, Cornell University, Johns Hopkins University, National Institute on Aging (NIA) at Harbor Hospital, Queens Medical Center, University of Pennsylvania, Oregon Health & Science University, and University of Texas at Houston. In addition to the GCCs, GenTAC will utilize two core groups. The Imaging Core is led by MedStar Health and the Phentoyping Core is led by Johns Hopkins University. During the first phase of the GenTAC Registry, 2296 subjects were enrolled. During the second phase, an additional 1,500 patients will be enrolled. Subjects enrolled will have data collected through medical record abstraction, physical examination, patient interview, and abstraction of other source records such as radiologic and echocardiography reports. Each subject will have an image sent to the Imaging Core for central reading. Blood and tissue samples will be sent to the RTI biospecimen repository. A project website will serve as the portal for communications, data entry, data query, reporting, and dissemination of information. Secure pages will be available only to authorized users. A portion of the website will include public pages targeted to study subjects and outside investigators. Registry activities will be directed and monitored by the NHLBI and the Steering and Operations Committees through regular conference calls and in-person meetings. In addition, an NHLBI-appointed Observational Study Monitoring Board (OMSB) will perform independent monitoring of overall progress, clinical outcomes, and patient safety. The Registry includes a policy for sharing data and specimens that will permit Registry investigators and researchers outside the Registry to request these resources for research purposes.

1.0 Background and Rationale

Thoracic aortic aneurysm (TAA) is a life-threatening condition and the underlying cause of almost 2,000 deaths in the United States every year (U.S. DHHS 2005). TAA, defined as the dilatation of any part of the thoracic aorta, is associated with high rates of mortality, morbidity, and surgical procedures. TAA can involve the ascending aorta, arch, or descending aorta (Hasham et al., 2002). Aortic aneurysms result from degeneration of elastic fibers and collagen in the extracellular matrix of the connective tissue, which may be a response to hypertension, trauma, inflammatory disease, or genetic disorders of the connective tissue (Flachskampf, 2000; Hasham et al., 2002). While hypertension is the most common cause of TAA in the elderly, genetic disorders, such as the Marfan syndrome, are more often the cause of morbidity and mortality from TAA in people who are young or middle aged (Flachskampf, 2000; Sisk et al. 1983; Tayel et al., 1991). The population prevalence of genetically-associated TAA is approximately 1 per 1,000 (Devereux unpublished data). Medical advances over the last 30 years have provided dramatic improvements for individuals with genetically triggered aneurysms (Silverman et al., 1995). However, even today, the burden of premature morbidity and mortality is profound.

Marfan syndrome (MFS) is a genetic disorder of the connective tissue that is associated with an extremely high risk of a TAA at young and middle ages (Elefteriades, 2004; Hwa et al., 1993; Groenink et al., 1998a). The most common sources of premature mortality associated with MFS and other causes of TAA are acute dissections or ruptures. Left untreated, nearly 25% of patients with TAA dissections will die within 24 hours while 75% will die in 2 weeks (Moore et al., 2002; Chen et al., 1997). Surgical procedures for acute ruptures and dissections also have high rates of mortality (Mehta et al., 2002; Ehrlich et al., 2000; Gott et al., 2002; Clouse et al., 2004). In a case series of more than 400 acute aortic dissections occurring in six different countries, 27% of patients died within 30 days. In contrast, in a case series of 235 elective aortic root replacement surgeries among MFS patients, there was no 30-day mortality (Gott et al., 2002). Close monitoring of patients in order to undergo elective surgery prior to dissection has become standard practice for patients with TAA (Elefteriades, 2004; Clouse et al., 2004).

While multiple other genetic conditions are associated with a significant risk of thoracic aortic aneurysm and dissection, far less is known about their etiology, pathogenesis and optimal medical and surgical management. Bicuspid or bicommissural aortic valve (BAV) is the most common form of congenital heart disease, occurring in about 1-2% of the population. A significant albeit undetermined percentage of individuals with this condition is at risk for ascending aortic enlargement with a predisposition for aortic tear or rupture (Borger and David, 2005; Holmes et al., 2007; Ortiz et al., 2006). Unlike Marfan syndrome, where maximal aortic dilatation tends to occur at the sinuses of Valsalva, most patients with BAV show maximal dilatation above the sinotubular junction in the more distal ascending aorta. While a small subset of people with BAV and aneurysm harbor heterozygous mutations in *NOTCH1* (Garg et al., 2005), the cause of this condition in most affected individuals remains unknown. Additional open questions include the utility of prescribing medications to decrease hemodynamic stress, the optimal timing for surgical intervention, and the surgical approach that should be utilized. Some patients that undergo repair of an isolated ascending aortic aneurysm have shown later dilatation of the aortic root, raising the question of whether replacement of the entire ascending aorta should be recommended at the time of the first operation. Additional controversy relates to the use of the Ross procedure, in which the pulmonary artery in used to replace a dilated ascending aorta. Concerns have been raised based upon the observations of pulmonary artery dilatation and/or architectural abnormalities of the pulmonary artery wall in patients with BAV/TAA, and reports of structural failure of the neoaorta when the Ross procedure has been performed for patients with BAV/TAA or other connective tissue disorders (Luciani et al., 2003).

Loeys-Dietz syndrome (LDS) is a dominant connective tissue disorder caused by mutations in either of the two genes that encode the TGFβ receptor (*TGFBR1* and *TGFBR2*) (Loeys et al., 2005; Loeys et al., 2006). While this condition shows some overlap with Marfan syndrome (pectus deformity, scoliosis, arachnodactyly, aortic root aneurysm and dissection) there are other discriminating features (e.g. hypertelorism, bifid uvula, craniosynostosis, cervical spine instability, translucent skin, easy bruising, dystrophic scarring, congenital heart disease, arterial tortuosity and a high risk of aneurysms and dissections throughout the arterial tree). Most importantly, aneurysms can tear or rupture at ages and vascular dimensions that do not confer risk in other connective tissue disorders such as Marfan syndrome. Because of the aggressive and less predictable course of vascular disease in LDS, some centers advocate early and aggressive surgical intervention and the use of medications such as beta blockers or losartan to reduce hemodynamic stress or antagonize TGFβ signaling, respectively. The impact of such measures on outcome remains to be determined.

Vascular Ehlers-Danlos syndrome (vascular EDS) is a systemic connective tissue disorder caused by mutations in type III collagen (Pepin et al., 2000). Affected individuals are at risk for life-threatening manifestations including visceral rupture and dissection or rupture of any medium-to-large sized muscular artery, with or without prior dilatation. Prophylactic surgery is strictly avoided in this population due to severe tissue friability and a high rate of life-threatening surgical complications. Precise scenario-dependent estimation of the risk-benefit relationship for surgical intervention or endovascular procedures is lacking.

Turner syndrome is a relatively common chromosomal abnormality (X0 karyotype) that can be associated with multiple vascular abnormalities including BAV, ascending aortic aneurysm and dissection and coarctation of the aorta (Hirose et al., 2000; Weytjens et al., 2000; Lin et al., 1998). The proportion of people with Turner syndrome at risk for aortic aneurysm requires further definition. Indeed, the definition of aortic aneurysm in this population is problematic given their small body size. Current sized-based definitions may overly penalize this patient group with practical implications including the prescription of exercise restrictions in a population at risk for obesity and hypertension. It is likely that evidence-based medical and surgical management guidelines for those with unequivocal aortic enlargement will derive from this Registry.

1.1 Genetic Screening and Family Pedigrees

MFS is related to mutations in the fibrillin-1 gene (*FBN1*) (Dietz et al., 1991; Maslen et al., 1991). Currently, more than 500 *FBN1* mutations that cause MFS or related conditions have been described (Collod-Beroud, 2002). In fact, most Marfan-associated *FBN1* mutations are ‘private’, with a different mutation found in virtually every affected family. Since similar genotypes may have different phenotypic expression, identifying mutations does not necessarily have prognostic utility (Dietz, 2003). Patients with the same mutation may show wide variation in clinical severity (Dietz et al., 1992). Only mutations clustered in the protein region encoded by exons 24-32 have shown a genotype-phenotype correlation; these mutations may be associated with neonatal MFS and they are more likely to be sporadic than to run in families (Collod-Beroud and Boileau, 2002).

MFS mutations are inherited in an autosomal dominant fashion with complete penetrance at *FBN1*. In 75% of cases, at least one parent has MFS. However, the family history may appear to be negative because the parent’s condition may not be diagnosed. About 25% of cases have a *de novo* gene mutation. Germline mutations also occur (Collod-Beroud et al., 1999; Rantamaki et al., 1999). In this case the parent is clinically unaffected, but still able to transmit the trait to offspring.

Although genetic screening can be used to aid in diagnosis in some cases where linkage is possible, comprehensive mutation screening fails to identify a mutation in 10% to 30% of those who meet established diagnostic criteria for MFS (Dietz, 2003). This may suggest genetic heterogeneity, and the identity of a second Marfan-like locus (MFS2) has recently been discovered (Mizuguchi et al. 2004). In this study, it was shown that heterozygous inactivating mutations in the TGFβ receptor-2 gene (*TGFBR2*) cause a Marfan-like syndrome in some individuals.

There is, however, controversy as to whether *TGFBR2* mutations result in MFS, or a related but distinct disorder. A recent study of 10 families with ascending aortic aneurysms and dissection as part of a more complex phenotype revealed heterozygous inactivating mutations in either *TGFBR2* or *TGFBR1* (Loeys et al., 2005). The phenotype overlaps with MFS, but these authors conclude that it is a distinct disorder (Loeys-Dietz syndrome, in which ectopis lentis has never been described), whereas Mizuguchi et al. described their patients with *TGFBR2* mutations as having MFS. With regard to TAA, the important distinction made by Loeys et al. is that individuals with *TGFBR1* or *TGFBR2* mutations have more aggressive aneurysms that tend to rupture at an early age, whereas *FBN1*-associated aneurysms in MFS are more predictable with risk of rupture closely correlated with aortic root diameter.

Options for molecular diagnosis of MFS and related disorders are limited due to the complications of genetic and allelic heterogeneity, the attendant difficulties in interpretation, and inability to identify FBN1 mutations in some MFS families with unequivocal linkage to FBN1. Protein-based methods for the molecular diagnosis of MFS may offer promise for both diagnostic and prognostic assessments since they reflect gene expression and not genotype. However, these techniques are still being established (Aoyama, 1995; Dietz, 2003). Development of improved diagnostic methods would benefit immensely from the resources made available by the GenTAC Registry.

1.2 Treatment

Life expectancy with MFS has risen dramatically in the last 30 years. In 1972, the median probability of cumulative survival was 48 years, but by 1993, it had risen to 72 years (Silverman et al., 1995). Surgical innovations appear to be responsible for the bulk of this improvement in MFS and other TAA patients (Clouse et al., 2004; Silverman et al., 1995; Gott et al., 2002). Beta-blockers have also contributed to the prevention of acute dissections, the principle cause of early mortality from MFS (Silverman et al., 1995), through reduction in blood pressure which slows the progression of aortic dilation.

An open label randomized trial of propanalol in 70 patients observed a significantly slower rate of progression in patients taking beta blockers than in controls as well as a significant decrease in the rate of aortic complications over a 10 year period (Shores et al., 1994). Further support for use of beta-blockers has been provided by two non-randomized studies conducted in young individuals. Rossi-Foulkes et al. (1999) found slower aortic dilatation, especially when related to body growth, in children and adolescents receiving treatment, predominately with beta-blockers, compared to untreated Marfan patients. Ladouceur et al. (2007) compared age-matched groups of 78 and 77 children who did or did not receive beta-blocker therapy and found that, in multivariate analysis, beta-blocker treatment was associated with a significantly decreased rate of sinuses of Valsalva dilatation. The actions of beta-blockers to reduce cardiac contractility and hence arterial dP/dt and to reduce the number of heart beats per day that impose stress on the aorta have been considered to contribute to aortic protection. Serial echocardiograms in 945 hypertensive patients showed greater reduction throughout a 5-year treatment period of the velocity of left ventricular fiber shortening, a measure of cardiac contractility, by beta-blocker therapy than by alternative antihypertensive therapy with equal blood pressure lowering (Bella et

al.: 2006). In a small sample of hypertensive patients, other drug classes including calcium channel agonists, angiotensin converting enzyme inhibitors, and angiotensin receptor blockers were more effective at reducing vascular stiffness than beta blockers (Resnick, 2002). Moreover, the appropriateness of the use of beta blockers in children with MFS who do not have aortic dilatation has not been evaluated but given the hemodynamic effects of these drugs and the lack of elasticity in the thoracic aorta of children with MFS, this therapy may make sense where not contraindicated (Jeremy et al., 1994).

Recent evidence has highlighted the potential benefit of treatment of individuals with Marfan syndrome (and perhaps other genetically-determined forms of aortic aneurysm) with angiotensin II receptor blockers (ARBs) such as losartan (Habashi et al., 2006). To date, the bulk of this experience derives from studies using a mouse model of Marfan syndrome harboring a missense mutation in the *Fbn1* gene. As in other tissues, there was evidence for increased TGFβ signaling in the aortic wall in fibrillin-1 deficient mice including increased nuclear accumulation of phosphorylated Smad2 (pSmad2), an effector of TGFβ signaling, and increased output of TGFβ responsive gene products such as connective tissue growth factor and collagens. Systemic administration of TGFβ blocking antibodies slowed aortic root growth over time and improved aortic wall architecture. Prior evidence had suggested that ARBs can antagonize TGFβ signaling through inhibition of expression of TGFβ and its receptor and also of activators of TGFβ such as thrombospondin-1 (Zhou et al., 2006; Naito et al., 2004). In a second trial in the mouse model, losartan-treated fibrillin-1 deficient mice could not be distinguished from wild-type mice by any parameter including aortic root growth, final aortic size, and aortic wall thickness and architecture (Habashi et al., 2006). These results were particularly dramatic given that therapy was not initiated until 2 months of age, after the establishment of aortic aneurysms. On this basis, a large NIH-sponsored multicenter clinical trial of losartan for patients with Marfan syndrome has been initiated. Losartan therapy may also prove relevant to other vascular disorders that have been associated with increased TGFβ signaling including Loeys-Dietz syndrome (Loeys et al., 2005), arterial tortuosity syndrome (Coucke et al., 2006) and autosomal dominant cutis laxa (Hanada et al., 2007).

Monitoring of patients with TAA is critical to prevent acute dissection. Flaschkampf (2000) identified transesophageal echochardiogram (TEE) as the fastest, safest, and most widely available means of securing information needed to decide whether a patient with TAA needs preemptive surgery. Computed tomography (CT) and magnetic resonance imaging (MR) are often used to supplement interpretation when TEE produces ambiguous findings. Transthoracic echocardiography is less effective for monitoring TAA, with a sensitivity of only 50% to 60% for dissecting lesions (von Kodolitsch et al., 1998; Nienaber et al., 1993). Long-term follow-up of patients in the GenTAC Registry may afford opportunities to better characterize the stage at which preventive surgery is optimal and to describe the characteristics of those requiring surgery sooner rather than later.

Operative and post-operative mortality has sufficiently improved to make elective aortic root replacement standard practice. Gott et al. (2002) estimated the 20 year survival in MFS patients with elective surgery for aortic root replacement done at Johns Hopkins to be 67%. Similar levels of survival were noted in a more diverse group of patients undergoing aortic root replacement at the Mayo Clinic (Zehr et al., 2004).

In patients with aortic aneurysm but normal aortic valve leaflets, valve sparing techniques have recently been introduced (Sarsan and Yacoub, 1993; David and Feindel, 1992; Underwood et al., 2000). However, in a small case series of patients undergoing a valve sparing procedure, 37% had recurrent aortic insufficiency with 28 months (Luciani et al., 1999), and in second a case series, recurrent aortic insufficiency was 26% at 5 years (Bethea et al., 2004).

The development of endovascular technology represents a major advance in the treatment of aortic aneurysms. Compared to traditional open aortic operations, less-invasive endovascular approaches are associated with significant reductions in early morbidity and mortality. However, the proper role of endovascular repairs in treating patients with genetically triggered aortic disease is unknown. Understandably, there are substantial concerns regarding the durability of endovascular repairs in patients with Marfan syndrome and other connective tissue disorders (Ince et al., 2005). For endovascular repairs to be successful, satisfactory fixation zones between the stent-graft and the aortic wall must be created and maintained. Because Marfan syndrome affects the entire aorta, available fixation zones are invariably diseased and, therefore, prone to future dilatation. When a fixation zone increases in size, the seal at the attachment site is eventually lost, subjecting the patient to dangerous complications such as endoleak, device migration, device erosion, and the need for reoperation to remove the device and re-repair the aneurysm. Endografts, however, may be well-suited for specific problems in these patients, such as for excluding focal pseudoaneurysms when an existing aortic graft can be used for the proximal and distal landing zones (Milewicz, 2005). Concerns regarding the use of endovascular stent grafts in Marfan patients naturally extend to include patients with other connective tissue disorders and may be also relevant to other forms of genetically triggered aortic disease. Long-term studies are needed to determine the appropriate circumstances for using endovascular stent grafts in these patients.

1.3 Etiology and Pathogenesis of Genetic Thoracic Aortic Aneurysms

Increased TGFβ signaling in the aortic wall has now been observed in both mice and people with multiple aortic aneurysm conditions including Marfan syndrome, Loeys-Dietz syndrome, arterial tortuosity syndrome, and autosomal dominant cutis laxa (Loeys et al., 2005; Habashi et al., 2006; Coucke et al., 2006; Hanada et al., 2007). While the etiologies and early pathogenetic events are quite distinct, the emerging view is that increased TGFβ signaling may represent a common final pathway to aneurysm formation. In Marfan syndrome, current evidence suggests loss of the normal ability for fibrillin-1 to sequester and inhibit the activation of the large latent complex of TGFβ (Neptune et al., 2003); in Loeys-Dietz syndrome excess signaling apparently manifests undisclosed but unproductive compensatory events initiated by heterozygous loss-of-function mutations in the *TGFBR* genes (Loeys et al., 2005); in arterial tortuosity syndrome complete loss of function of Glut10, a putative glucose transporter, associates with loss of expression of decorin, an extracellular inhibitor of TGFβ (Coucke et al., 2006). Histologic analysis of the aortic wall from patients with aneurysms, including those with *FBN1* and *TGFBR* mutations, show the hallmark features of “cystic medial necrosis”, a pathologic designation used to describe fragmentation and disarray of elastic fibers with the accumulation of amorphous matrix between fibers. The aortic wall from individuals with Marfan syndrome show increased collagen deposition, a finding that is accentuated in individuals with TGFBR mutations (Loeys et al., 2005). Given the commonality of multiple findings with potential pathogenetic relevance, the basis for the more widespread and severe vascular involvement in selected conditions (e.g., Loeys-Dietz syndrome) remains to be elucidated. In theory, the spatially-restricted disease seen in Marfan syndrome might reflect a predominant role for fibrillin-1 in regulating TGFβ signaling in the proximal aorta, with redundancy in this function in other aortic segments, while the widespread vascular disease in Loeys-Dietz syndrome might reflect the diffuse expression and nonredundant role of the TGFβ receptor in TGFβ signaling. Ultimately, the study of these mechanisms may lead to an improved understanding of the structural and material properties of the connective tissue of the vasculature. This may allow the development of disease-specific therapeutic strategies and of better synthetic materials to produce grafts and prosthetic valves. These studies may also have relevance to other disease states. For example, definition of the mechanism of muscle hypoplasia in Marfan

syndrome lead to the observation that excess TGFβ signaling also suppresses muscle regeneration in a mouse model of Duchenne muscular dystrophy (Cohn et al., 2007). Losartan therapy showed the ability to maintain muscle mass and strength in this mouse model.

One of the more challenging aspects of MFS is the fact that the same mutations can be expressed differentially in different members of the same family. This variable expression suggests that other parts of the genome such as genes controlling elastin or collagen formation may influence the extent of manifestations observed. This appears to be an especially provocative area for research that would be best facilitated by a Registry containing family members with varying levels of severity of MFS.

Although the majority of TAA patients do not have a genetic syndrome, like MFS or LDS, it is well established that genetic predisposition plays a role in the etiology of non-syndromic TAA, with least 20% of these patients have a family history of TAA. In these families, TAA is inherited in an autosomal dominant manner with decreased penetrance and variable expression Milewicz et al. (1998). A few familial cases of non-syndromic TAA have been linked back to the *FBN1* gene, but this situation is considered to be rare (Francke et al., 1995; Milewicz et al., 1996; Hasham et al., 2002). Using individual large families with multiple affected members, four loci for this disorder have been identified, TAAD1 on chromosome 5q13-q14 (Guo et al., 2001), FAA1 on chromosome 11q23.2-q24 (Vaughan et al., 2001), TAAD2 on 3p24-26 (Hasham et al.), and a locus for TAA associated with patent ductus arteriosus on 16p. The defective gene at the TAAD2 locus has been identified as the TGFBR2 gene (Pannu et al, 1995). In addition, the gene for TAA associated with PDA has been identified as MYH11. TGFBR2 and MYH11 account for less that 5% of the non-syndrome familial TAAD. Therefore, the progress on identifying genes predisposing to TAAD has been slow due to the genetic heterogeneity associated with the condition and the premature deaths of affected individuals in families. Identification of the genes involved in TAA/TAAD will be critical to determining the final common pathway underlying aortic aneurysms.

Data collected as part of GenTAC can be used to develop and evaluate improved methods for diagnosis, prognostic assessment and ongoing monitoring, to better understand the mechanical and structural properties of healthy and genetically variant connective tissue, and to improve surgical and pharmacological interventions. The Registry will facilitate progress in each of these research areas by providing a research population of adequate size whose clinical characteristics are well established, and by collecting detailed information on family history and biospecimens for genetic and molecular analyses. The patients enrolled in the GenTAC Registry may also serve as a source of patients to undergo randomized clinical trials to evaluate new therapies and participate in epidemiological studies that complement basic research into the molecular mechanisms of connective tissue disease. Ultimately, improved understanding of these molecular mechanisms may help generate new treatment strategies not just for those with genetically-associated TAAs, but also for other persons with apparently sporadic TAAs.

2.0 Objectives

The overall objective of GenTAC is to establish a Registry of patients with genetically induced thoracic aortic aneurysms and related conditions, and collect associated medical data, as well as blood and tissue samples. The Registry will establish a biospecimen inventory and bioinformatics infrastructure that will enable research to determine best medical practices to advance the clinical management of genetic thoracic aortic aneurysms, and other cardiovascular complications. To achieve these goals the Registry will:

1) Develop standard methods to collect data and specimens which will be used for research to characterize patients at risk for aortic rupture and dissection, and development of other cardiovascular complications.

2) Record the characteristics of patients undergoing surgical repair of aneurysms and their clinical outcomes
3) Process tissue and blood specimens
4) Analyze data collected
5) Provide access to these resources to Registry investigators and outside researchers who are interested in advancing the fundamental understanding of genetic aortic aneurysms and management of afflicted patients
6) Publish and disseminate results.

3.0 Research Questions

The Registry will either directly address or facilitate ancillary studies that will address the following key research questions that were developed during the first funding period:

QUESTIONS TO BE ADDRESSED BY GENTAC:

1) What are the phenotype-specific indicators for surgery for the inclusion criteria?
2) What surgical procedures provide the best clinical outcome?
3) Are there differences in surgical outcomes between Marfan and other TAA patients?
4) How do the new and old Ghent criteria compare in the study population?
5) What outcomes or complications are related to aging?
6) What is the disease progression in the Losartan naive population (who may or may not have been treated by β-blockers or other agents)?
7) What are the outcomes by inclusion diagnosis and organ system?
8) Do the rare examples of childhood dissection in MFS actually represent individuals with Loeys-Dietz who have been misdiagnosed?
9) What are the criteria for surgery?
10) Is the absolute size or rate in change in the dimension of the aortic annulus (sinotubular junction) a reasonable predictor of future valve function?
11) What are the anatomic indicators of impending dissection or other adverse outcomes?
12) What is the optimal frequency of follow-up?
13) What medications minimize disease progression?
14) What medications provide the best quality of life?
15) Describe the full spectrum of Loeys-Dietz syndrome.
16) What are the anatomic predictors of dissection based on imaging?
17) What are the anatomic predictors of response to therapy?

FUTURE QUESTIONS TO BE ANSWERED BY GENTAC WITH ADDITIONAL FUNDING:

18) Does C-reactive protein or other markers of acute inflammation have prognostic significance for impending dissection?
19) Does monitoring of fibrillin-1 degradation products aid in the monitoring of the progression of arterial wall damage irrespective of etiology of an aneurysm?
20) Does the amount of circulating biomarkers, like TGF-β, correlate with the disease severity?
21) Does increased TGFβ signaling correlate with increased expression and activation of matrix-degrading enzymes such as matrix metalloproteinase (MMP) 2 or 9 in aneurysms other than Marfan and Loeys-Dietz?
22) Are there genetic modifiers of vascular disease that influence the phenotypic-genotypic correlations among families and unrelated individuals?
23) What is the phenotypic variation among individuals that harbor the same mutation?
24) What are the pathogenetic mechanisms that contribute to aortic aneurysm in conditions such as bicuspid aortic valve with aneurysm and familial thoracic aortic aneurysm?

Additional funding was obtained to implement phase II of the Registry, which will spur additional research questions from participating investigators.

4.0 Registry Design

The GenTAC Registry is a longitudinal cohort study, which is observational by design. The cohort will consist of patients with conditions related to genetically-induced thoracic aortic aneurysms. There will not be a disease-free control or comparison group enrolled in the Registry. The study will compare cross-sectional and longitudinal data on risk factors related to the diagnosis, treatment and outcomes among groups of enrolled patients. As part of the natural course of clinical care, the patients and their physicians will determine the approach to treatment and the study will record the observed related outcomes. The study will not attempt to interfere with the outcomes through any type of planned intervention; therefore, there are no anticipated adverse events as a result of study participation.

4.1 Organization and Participating Institutions

The following sections describe the organizations that are participating in the GenTAC Registry including the study sponsor, data coordinating center, study chair, the eight GenTAC clinical centers (GCCs) and two core laboratories.

Sponsor

The National Heart Lung and Blood Institute (NHLBI) and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) are co-sponsors of the Registry. NHLBI manages the GenTAC Registry contract and has technical oversight and will monitor the expenditures and deliverables. The NHLBI and the investigators have a shared responsibility for the scientific direction and performance of the Registry.

Data Coordinating Center

RTI International will serve as the DCC for the GenTAC Registry (Dr. Barbara Kroner, PI). As the DCC, RTI is responsible for the data management, coordination of training, logistics and communications, study website, and statistical design and analysis. In concert with the GenTAC Steering Committee, the DCC is responsible for development of the protocol, data collection forms, manual of operations, and preparation of manuscripts and presentations.

Study Chair

The Study Chair, Dr. Kim Eagle, will be responsible for the conduct of the Registry Steering Committee and Operations Committee meetings and ensures that consensus is built and decisions are made in a timely manner. In collaboration with the DCC PI, the Chair will provide scientific and administrative oversight of the Registry and contribute to decisions regarding the inclusion of database elements and the analyses of relevant outcomes. The Chair will help author the policies and procedures that will be found in the Manual of Operations and will ensure that the deliverables agreed to in the contract are written in such a way to reflect the goals and purposes of the Registry.

Imaging Core (ICORE)

The Cardiovascular Core Laboratory at Medstar Research Institute was founded approximately twelve years ago and will serve as the Imaging Core Lab (ICORE) for GenTAC. The ICORE will be lead by Dr. Federico Asch (PI) and Dr. Neil Weissman (Co-PI). Medstar has served as the Imaging Core Lab for more than 100 multicenter clinical trials involving over 50,000 exams from more than 500 hospitals internationally. The Core Laboratory facilities occupy over 3,200 square feet of office space within the campus of the Washington Hospital Center in Washington, DC. The Core Laboratory is equipped with dedicated servers and review stations. These include: Philips Xcelera, Digisonics DigiView, Indec, and Medis workstations. Medical software and devices used in the Core Laboratory are 510K approved. The GenTAC ICORE will be responsible for finalizing the image acquisition protocols and case report form as well as the receipt, tracking, reading, overreading, and storage of all de-identified images sent from the GenTAC clinical centers.

Phenotyping Core (PCORE)

Johns Hopkins University will serve as the GenTAC Phenotyping Core (PCORE) under the direction of Dr. Jennifer Habashi. Johns Hopkins University is one of the largest and most prestigious institutions in the world for the diagnosis and treatment of patients with genetically induced thoracic aortic aneurysm and connective tissue disorders. The PCORE will establish the data algorithms for validating the confidence level of the eligibility diagnoses assigned to GenTAC subjects. They will work with the DCC to program the algorithms, review and interpret the results, and determine when the DCC should request additional information from the GCCs .

GenTAC Clinical Centers (GCCs)

The GenTAC Registry population will be comprised of patients from eight GenTAC Clinical Centers GCCs, which provide care to patients from a wide geographic catchment area in the Northeast, Middle-Atlantic, South and Northwest plus the island of Hawaii. The participating GCCs include:

- Baylor College of Medicine (Dr. Scott LeMaire, PI)
- Johns Hopkins University School of Medicine (Dr. Kathy Holmes, PI)
- NIA at Harbor Hospital (Dr. Nazli McDonnell, PI)
- Oregon Health & Science University (Dr. Cheryl Maslen, PI)
- Queens Medical Center (Dr. Ralph Shohet, PI)
- University of Pennsylvania Health System (Dr. Reed Pyeritz, PI)
- Weill Medical College of Cornell University (Dr. Richard Devereux, PI)
- University of Texas Medical School at Houston (Dr. Dianna Milewicz, PI)

Baylor College of Medicine (BCM), and its seven affiliated teaching hospitals (including St. Luke's Episcopal Hospital and Texas Children's Hospital) enjoy international reputations for medical excellence. BCM has been a primary partner with UT in the GenTAC. BCM physicians

see more than 135,000 inpatients and more than 1.7 million outpatients each year in affiliated hospitals and clinics. Patients are admitted from all 50 states and from around the world.

Johns Hopkins University School of Medicine (JHU). The Medical Genetics Clinic at the Institute of Genetic Medicine at the Johns Hopkins University is one of the largest and most prestigious institutions in the world for the diagnosis and treatment of patients with genetically induced thoracic aortic aneurysm and connective tissue disorders, including MFS. The Connective Tissue Clinic is a multidisciplinary clinic for the evaluation and management of individuals with connective tissue disorders that affect the cardiovascular system. The Clinic currently follows more than 450 patients, including those with significant aortic enlargement that are not yet candidates for surgical repair, those for whom surgery is anticipated in the near future, and those who have already undergone cardiovascular surgery for genetically induced thoracic aortic aneurysm. Additionally, the clinic sees more than 200 new patients annually with MFS and other genetic disorders of the aorta. Johns Hopkins is unique in its emphasis on children with connective tissue disorders affecting the heart. While patients in all age groups are represented, from birth to old age, approximately two-thirds of the patients are less than age 18. This will provide an important and unique opportunity to document the natural history of these conditions in children, to document their response to therapy, and to derive insight that will be needed to prevent rather than repair manifestations of disease.

NIA at Harbor Hospital (NIA). The National Institute on Aging maintains a cutting-edge clinical research unit located on the fifth floor of Harbor Hospital. In the medical genetics clinic, under the direction of Dr. Nazli McDonnell, more than 200 patients from around the world are evaluated annually for EDS. As part of Dr. McDonnell's NIA-funded EDS research study, these patients receive a comprehensive evaluation, including genetic testing, medical examinations, and cardiovascular imaging studies, which may extend for 2 days or more. Many of these patients meet the eligibility criteria for the GenTAC Registry and can be recruited into the Registry during their visit to the NIA facility.

Oregon Health & Science University (OHSU). Oregon Health & Science University provides an excellent environment for recruitment of patients for the GENTAC Registry. The OHSU Division of Cardiothoracic Surgery has significant experience and expertise in the diagnosis and management of thoracic aneurismal disease. The Pediatric Cardiology Clinic at Doernbecher Children's Hospital on the OHSU campus and the OHSU Children's Center are the regional resource for the diagnosis and management of children with genetic disorders. There is a long history in the diagnosis and treatment of children and families with MFS, with over 250 children with MFS currently being cared for at these facilities. OHSU has a strong history in providing clinical outreach to rural areas in Oregon, Washington, Idaho, Montana, and Alaska. In particular, cardiac and genetic services from OHSU are networked throughout these regions, providing a large and diverse patient catchment area. Since the racial demographic of this extended catchment area is unusually high in American Indian, Alaska Native, and Pacific Islander populations, recruitment through the OHSU center will provide a unique demographic component to the GENTAC Registry.

Queens Medical Center (QMC). The Queen's Medical Center is a private, non-profit, acute medical care facility in Honolulu, Hawaii. As the leading medical referral center in the Pacific Basin, QMC offers a comprehensive range of primary and specialized care services. With the latest technologies in interventional cardiology and cardiac surgery, Queen's Heart at QMC is the most comprehensive program in Hawaii for the diagnosis and treatment of patients with thoracic aortic disease and its complications. The population demographics for patients with TAAD at Queen's Heart include 75% of Hawaiian, Pacific Islander, or Japanese descent.

University of Pennsylvania Health System (UPHS). The Division of Medical Genetics at the University of Pennsylvania is dedicated to the comprehensive evaluation and management of children and adults with MFS and related conditions. The Division provides coordinated multidisciplinary care including diagnosis, genetic testing, education, genetic counseling, management, and support. UPHS brings an impressive set of qualifications to the GenTAC Registry because of the relationship between several existing programs. The Thoracic Aortic Surgical Program at UPHS and follows over 1,200 outpatients annually and operates on over 300. This program represents one of the largest thoracic aortic clinical services in North America. The Division of Medical Genetics has one of the largest multidisciplinary clinics devoted to MFS in the United States. The division evaluates over 200 patients each year for the possible diagnosis of MFS or another connective tissue disorder. Many patients with aneurysms and dissections who do not have MFS are also seen at this clinic; these patients often have a family history of similarly affected relatives.

Weill Medical College of Cornell University (Cornell). The participation of Cornell University provides a unique opportunity to register patients from ethnically diverse populations cared for by both the New York Presbyterian Hospital and the Hospital for Special Surgery located in the New York City metropolitan area. On the New York Presbyterian Cornell campus alone, cardiologists see more than 100 new patients per year with MFS and other genetic disorders of the aorta. New patients are evenly divided between men and women and have a diverse ethnic distribution (predominantly African-American, Hispanic, Asian, and Caucasian) paralleling that of the broad New York metropolitan area. Cornell cardiothoracic surgeons operate on more than 100 patients per year with aortic disease with similar gender and ethnic distributions.

University of Texas Medical School at Houston (UT). The University of Texas Medical School at Houston registers patients from ethnically diverse populations through its affiliation with Memorial Hermann Hospital, the Memorial Hermann Healthcare System, and the Texas Medical Center. In addition, patients are referred both national and internationally for complex aortic surgical repair and genetic consultations. . The Medical Genetics Clinic at the University of Texas Medical School at Houston offers patients and family services for inherited cardiovascular disease, including MFS, familial aortic aneurysms and dissections, and strokes. The majority of patients are referred from Texas and the surrounding states, but patients will travel to Houston for evaluation from the Southwest and Southern regions of the United States.

During the first phase of GenTAC, 2296 subjects were enrolled. Under Phase II of GenTAC, the new recruitment targets are reduced and vary by site. Table 1 presents the target overall enrollment goals for GenTAC.

Table 1 – Target enrollment for GenTAC

	Original sites						New sites		
	COR	JHU	PEN	OHS	UT	BAY	NIA	QMC	TOTAL
No. enrolled GenTAC phase I	547	474	502	395	210	168	--	--	2296
GenTAC phase II Target	275	200	275	175	200	100	150	150	1525
TOTAL ENROLLMENT	822	674	777	570	410	268	150	150	3821

The enrollment targets were reduced from the first phase of GenTAC for a number of reasons, including:

- Concentration on conducting follow-up during Phase II of GenTAC,
- A focus on new patients that have complete phenotyping, have an image available for the ICORE, and will be available for follow-up, and
- An increase in other responsibilities at the GCCs.

The eight participating GCCs treat patients from different parts of the U.S., and from a mix of urban, suburban and rural environments. Hence, the Registry should be adequate to provide basic descriptive information on variation in health outcomes according to patient demographic and health characteristics with some information on impact of population density and regional differences. As important, since referral for diagnosis is likely to occur at the primary care physician level, the Registry may be able to detect regional variation in patterns of diagnosis, adequacy of monitoring, and frequency of preemptive versus emergency surgical procedures. The addition of two new sites, QMC and NIA into GenTAC Phase II, will enhance the Asian/Pacific Islander and the Ehlers-Danlos patient populations, respectively.

Biospecimen Repository

The NHLBI contract repository will provide the long-term storage of biospecimens. The repository will store all samples indefinitely or until the sample is used up. This storage procedure is stated explicitly in the patient consent. Patients may request in writing at any time that their samples be destroyed. All samples will be identified with a code number and not subject identifying information. The repository has no authority to distribute samples to any investigator without a requisition which will be generated by the DCC and must be approved by the NHLBI and the GenTAC Steering Committee.

RTI will serve as the specimen processing laboratory and short-term repository. DNA extraction and lymphocyte immortalization will also be performed. Testing laboratories (e.g., resequencing and biomarkers) will vary based on the needs of the Registry. For example, the NHLBI RS&G Service performed resequencing of 560 samples under the first phase of GenTAC.

4.2 Committees

The **Steering Committee** (SC) is comprised of the Principal Investigator from each GCC, the PI from the two Cores, the Project Officer from the NHLBI, the Principal Investigator from the DCC, and the Study Chair. The SC will act as the governing body of the GenTAC Registry and will establish the scientific direction, along with the other investigators at the GCCs and the Scientific Advisory Board. The SC will communicate by regular conference calls, e-mail and annual in-person meetings. Written minutes of meetings will be available within 30 days and posted on the project website.

The **Operations Committee** (OC) is comprised of the Study Chair, the NHLBI Project Officers and Clinical Trials Specialist, and the DCC PI and Senior Project Manager. The OC has oversight and authority for the day-to-day operations of the Registry and will meet by teleconference bi-weekly.

The **Scientific Advisory Board** (SAB) will provide an unbiased review of proposed research and guidance on approval of Registry resources to conduct these proposed projects. The SAB will be comprised of experts in the fields of medical genetics, cardiothoracic surgery, cardiology and industry specializing in the treatment of TAAD. Ad hoc members may be sought at times for review of some research proposals, if additional expertise is needed. NHLBI staff may not serve as reviewers on the committee but may participate in reviews to provide programmatic information.

The **Ancillary Studies Committee** (ASC) will develop a policy for the submission, review and approval of ancillary studies, as well as how to incorporate the SAB in strategic planning and the review of ancillary study proposals. The **Publications and Authorship Committee** (PAC) will review manuscripts and abstracts prior to submission and assure that appropriate authorship is presented. The **Imaging Committee** (IC) will develop procedures for transfer of imaging studies to the ICORE and will finalize the data to be read from each image. The **Phenotyping Committee** (PC) will establish the data items needed to create algorithms and verify diagnostic categories. The **Media and Promotions Committee** (MPC) will work with the SAB and other committees to promote the use of registry resources and subject retention.

Other committees will be formed as necessary.

4.3 Observational Study Monitoring Board (OSMB)

An NHLBI-appointed OSMB, comprised of a multidisciplinary group of experts, will perform independent monitoring of overall progress, clinical outcomes, and patient safety Registry. The OSMB will also make recommendations regarding appropriate protocol and operational changes. Any decision to modify the protocol or significantly change study operations may have a substantial effect upon the Registry. Thus, the OSMB will play an essential role in assuring quality research.

The principal role of the OSMB is to regularly monitor the data from the Registry, review and assess the performance of its operations, and make recommendations, as appropriate, to NHLBI with respect to:

- the performance of individual centers (including possible recommendation on actions to be taken regarding any centers that perform unsatisfactorily);
- issues related to participant safety and informed consent, including notification of and referral for abnormal findings;
- adequacy of study progress in terms of recruitment, quality control, data analysis, and publications;
- issues pertaining to participant burden;
- impact of proposed ancillary studies and substudies on participant burden and overall achievement on the main study goals;
- possible modifications in the study protocol; and
- overall scientific direction of the Registry.

The OSMB is composed of a Chair and members with expertise in biostatistics, cardiology, vascular surgery, bioethics, and genetics. It is anticipated that the OSMB will meet semi-annually via in-person meetings or teleconferences. Prior to these meetings, the Registry DCC will prepare and distribute semi-annual reports of recruitment, progress and data to the OSMB.

4.4 Inclusion and Exclusion Criteria

Inclusion Criteria

1. Patients diagnosed with one of the 12 conditions listed in the table below, and also meeting the requirements for age, aortic involvement and family history as indicated.

	Conditions Diagnosed	Age Range	Minimum Thoracic Aortic Enlargement Required	Family History Required
1	Marfan syndrome	All ages	None	No
2	Turner syndrome	All ages	None	No
3	Ehlers-Danlos syndrome	All ages	None for vascular EDS. Other types: 2 SD above normal based on nomograms[4] OR prior dissection OR significant enlargement compared to adjacent area	No
4	Loeys-Dietz syndrome	All ages	None	No
5	FBN1, TGFBR1, TGFBR2, ACTA2 or MYH11 genetic mutation[1]	All ages	None	No
6	Bicuspid aortic valve without known family history	All ages	2 SD above normal based on nomograms OR prior dissection OR significant enlargement compared to adjacent area	No
7	Bicuspid aortic valve with family history	All ages	None	Yes[2]
8	Bicuspid aortic valve with coarctation	All ages	None	No
9	Familial Thoracic Aortic Aneurysm and Dissections	All ages	2 SD above normal based on nomograms OR prior dissection OR significant enlargement compared to adjacent area	Yes[3]
10	Shprintzen-Goldberg syndrome	All ages	None	No
11	Other aneurysms and dissections of the thoracic aorta not due to trauma.	≤ 50 years of age	2 SD above normal based on nomograms OR prior dissection OR significant enlargement compared to adjacent area	No
12	Other congenital heart disease with aortic enlargement (ie.g., Tetralogy of Fallot, corarctation)	All ages	2 SD above normal based on nomograms OR prior dissection OR significant enlargement compared to adjacent area	No
13	1st degree family members of probands already enrolled in the GenTAC Registry [5]	All ages	None	Yes

[1] ***ACTA2*** and ***MYH11*** mutations causing thoracic aortic disease should be confirmed by a Gentac PI.

[2] **Family history for BAV** = First degree relative with thoracic aortic enlargement 2 SD above normal based on nomograms, prior thoracic aortic aneurysm repair or dissection, significant enlargement compared to adjacent area, BAV, or aortic coarctation.

[3] **Family history for FTAAD** = 1) First or second degree relative of any age with thoracic aortic enlargement 2 SD above normal or prior thoracic aortic aneurysm repair or aortic dissection OR 2) Nonsmoking (for >15 years) first degree relative ≤ 50 years of age with aneurysms or dissection of the abdominal aorta or any artery.

[4] Roman MJ et al. Am J Cardiol 64:507-512, 1989. See nomograms on the following pages.

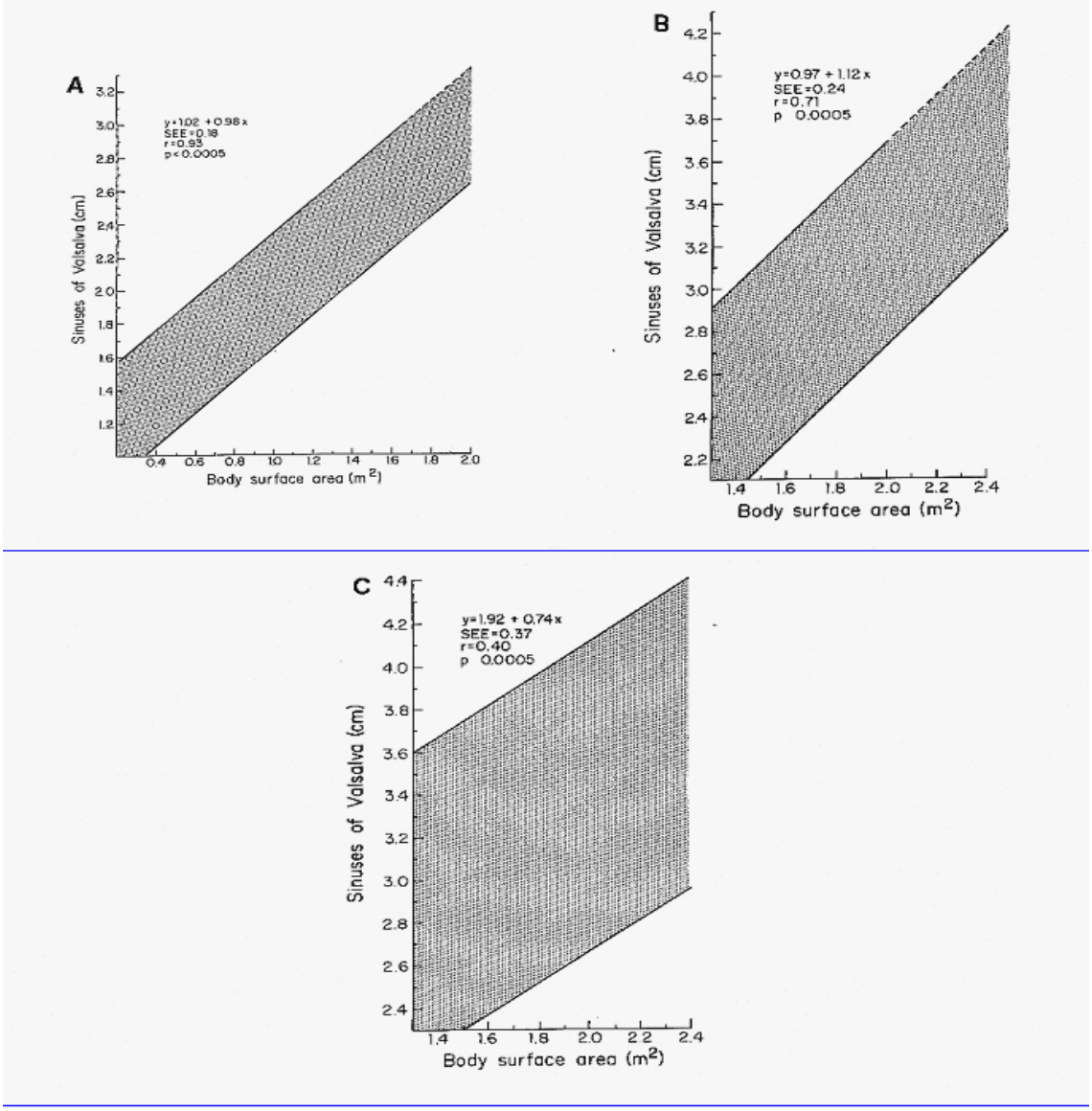
[5] Subjects were enrolled in this category in 2009 under special circumstances and with prior approval of the SC

2. All races and ethnicities for which informed consent can be obtained.
3. Ability of patient, parent or guardian to provide informed consent or parental consent.
4. All eligible patients regardless of follow-up potential (e.g., international, other referral)
5. All eligible patients regardless of willingness to complete the self-administered questionnaires.

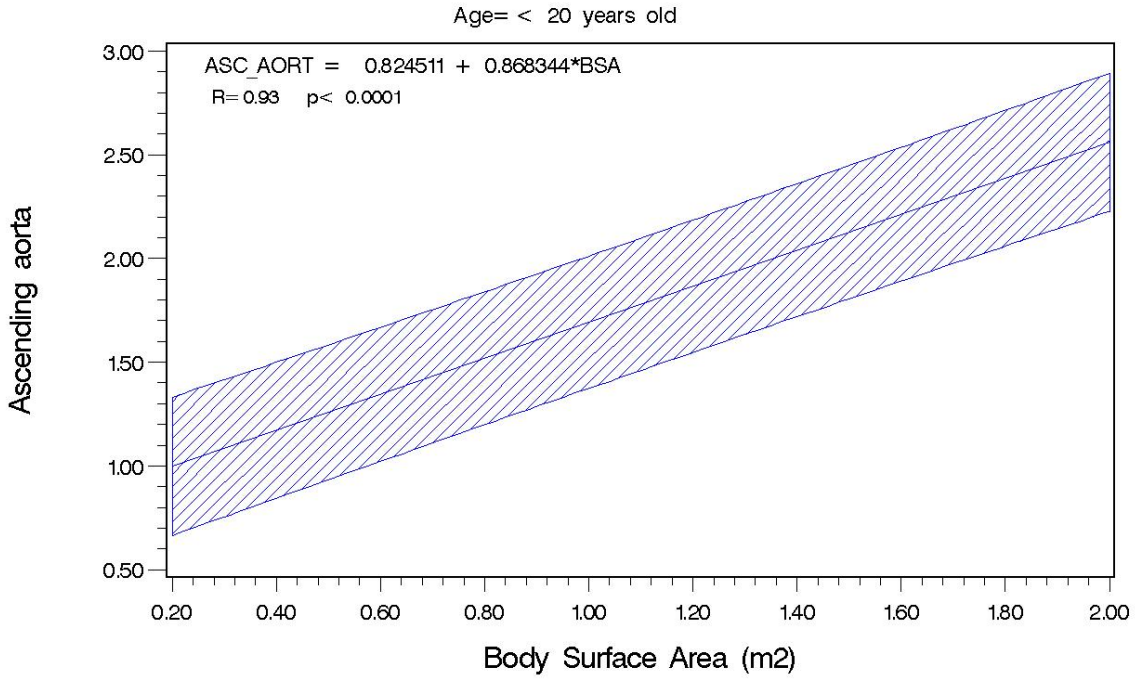
Exclusion Criteria

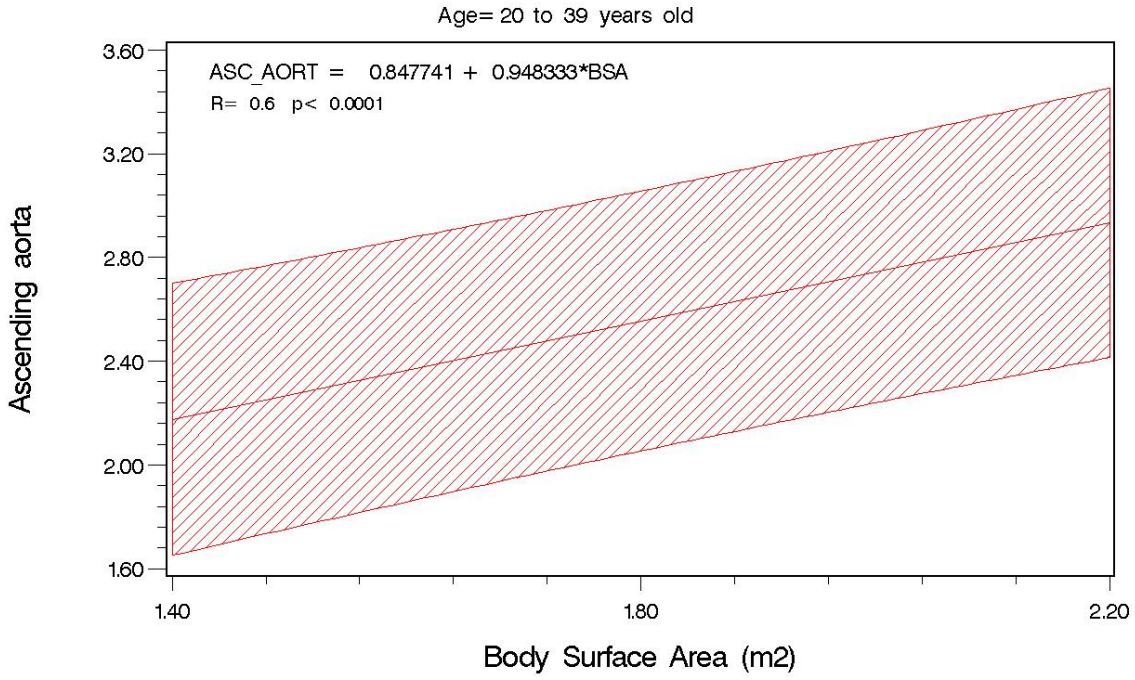
1. Inability of the patient, parent or guardian to give consent.
2. Unwillingness to provide a blood or buccal specimen.

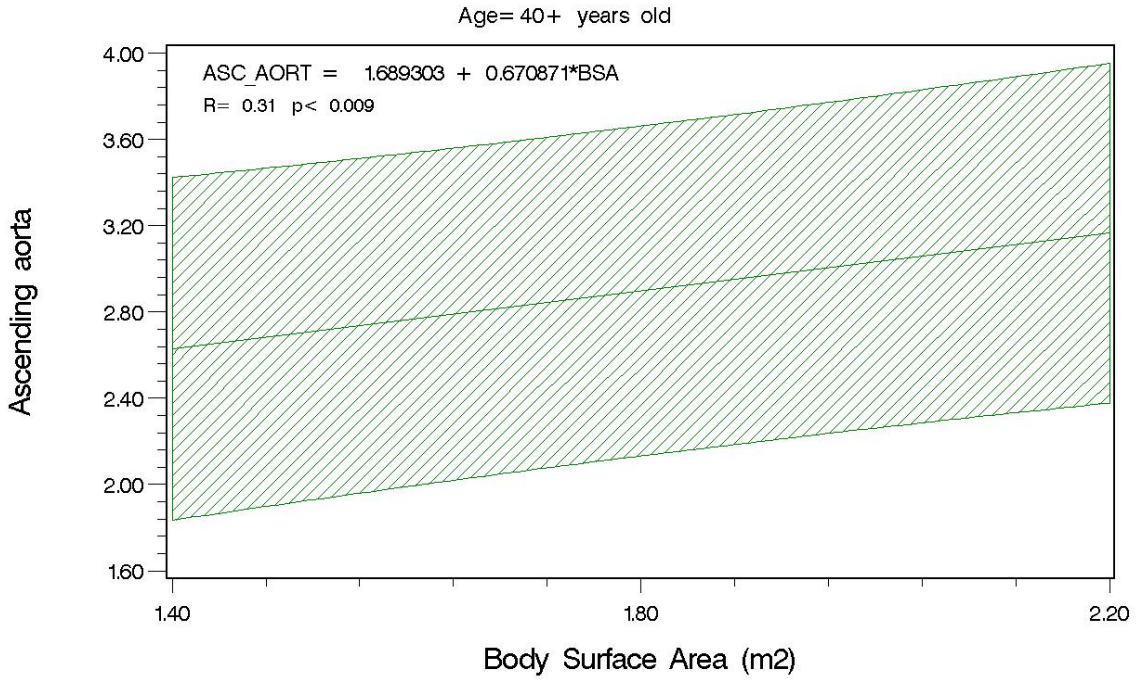
Nomograms for aortic root dilation by body surface area (BSA) for age groups < 20 years (A), 20-40 years (B) and >40 years (C)



Nomograms for <u>aortic root</u> dilation by body surface area (BSA) for age groups < 20 years, 20-40 years and >40 years







4.5 Recruitment and Consent

Information about the Registry will be disseminated to potential study subjects using a number of sources. Most notably will be the collaborations with the patient advocacy groups such as the National Marfan Foundation, Ehlers-Danlos National Foundation, and Turner Syndrome Society. These organizations have well established resources for providing information to patients such as websites, newsletters, and national conferences. A recruitment brochure has been developed to explain the goals and basic procedures of the Registry. Finally, the study website, http://gentac.rti.org will inform potential subjects about the Registry. All recruitment materials will be approved by the DCC and GCC IRBs, as appropriate, prior to dissemination.

GCC study staff will identify eligible patients using the eligibility criteria developed and approved by the Steering Committee. They will both screen their current patient population as well as identify new patients that attend the clinic. Eligible patients will be solicited during clinic visits according to protocols approved by the local IRB. Depending on the geographic area covered by the GCC, patients may also be recruited during outreach visits to outlying areas or through other outreach efforts within the community. This flexibility on the part of the GCC will insure inclusion of the greatest number of eligible patients for the Registry.

Initially, a care provider will introduce the Registry to the patient or the parent or guardian of the patient, and ask if they are interested in talking further with the research coordinator. If the patient (or parent or guardian) agrees, the research coordinator will meet with the patient for a more comprehensive explanation of the Registry. If there is continued agreement, the research coordinator will proceed with the consent and enrollment process. Signed informed consent will be obtained prior to any data or sample collection (Appendices A-C). Patients will receive a hard-copy of the consent form to keep. Patients will be able to ask questions at any time. One parent or guardian may provide signed consent for a minor child or other person with a physical or mental condition that prevents them from doing so themselves. Literacy in English or Spanish will be required of the consenting patient or parent/guardian. Child assent will be obtained from children at least 8 years of age, or as determined by the local IRB. A signed HIPAA Research Authorization is also required of all participants.

4.6 Project Website

The GenTAC website will serve as a source of information for both the general public and collaborators who are participating or wish to participate in the research effort. Its content will be closely monitored and kept up-to-date so that the most current information is available to visitors.

Public Website

The project website, http://gentac.rti.org, is available to the general public and will be used most notably to disseminate information about the Registry to potential subjects, enrolled participants, and outside investigators interested in accessing GenTAC data for ancillary studies. The public pages will include 1) project description and rationale, 2) participating institutions and contact information, 3) links to project newsletters, 4) study protocol, 5) instructions for requesting access to data and biospecimens, (6) list of publications and copies of abstracts, and 7) links to organizations which provide information and resources about the conditions under study.

Restricted Website

The restricted area of the study website will provide a secure, unified communication channel for all GenTAC activities and will consolidate access to the data capture and management systems, as well as maintain the repository of study documents. This ensures that all research staff have one place to access GenTAC information and study systems. Access to information and systems through the portal will be authenticated for users based on the definition of study roles. These roles allow access only to those systems needed by a particular user to carry out the responsibilities of his/her role. Role-based access protects data confidentiality and security and increases data integrity.

5.0 Data Collection

Patients enrolled in the Registry will be assigned a unique identification number by which they will be identified through the life of the Registry. The identification number will be assigned at the GCC by the research coordinator after a patient provides consent to participate in the Registry. The link between the patient and associated identification number will remain at the enrolling center under restricted access.

5.1 Enrollment Data

As part of the enrollment visit at the clinical center, a number of data forms will be completed. They include:

- Clinic Evaluation Form
- Enrollment Patient Questionnaire
- Family History Form
- Imaging Evaluation Form (when an image is not available to send to the Imaging Core)
- Non-Thoracic Imaging Evaluation
- Genetics Form
- Surgical and Percutaneous Interventions Form
- Tissue Collection Form

Clinic Evaluation Form (Appendix D)

This form will be completed by research staff for all patients at the time of enrollment into the Registry. It provides baseline information about the patient's health at the time of enrollment. Completion of this form will provide demographic and diagnosis information, anthropometric measurements, organ systems review, and treatment.

Enrollment Patient Questionnaire (Appendix E)

This self-administered form will be completed by the patient or consenting parent at the time of enrollment. Questions not applicable to children will be so indicated. Completion of this form will provide information about the patient's lifestyle, quality of life, and history of medications, other treatment, and personal diseases.

Family History Form (Appendix F)

This form will be completed by the patient with assistance from the research staff. Completion of this form will provide a pedigree and information about the disease history of family members. Protection of family health information and family members as secondary research subjects are discussed in Section 9.0

Imaging Evaluation Form (Appendix G)

This form will be completed by research staff to record relevant information from the most recent echo-cardiogram, CT scan or MRI when these studies are not available to send to the Imaging Core. If additional retrospective evaluations are of interest, additional forms may be completed for each unique evaluation date.

Non-Thoracic Imaging Evaluation Form (Appendix H)

This form will be completed by research staff to record relevant information from the most recent echo-cardiogram, CT scan or MRI of the head or other non-thoracic areas. If additional retrospective evaluations are of interest, additional forms will be completed for each unique evaluation date.

Genetics Form (Appendix I)

This form will be completed by research staff to record prior genetic testing.

Surgical and Percutaneous Interventions Form (Appendix J)

This form will be completed by research staff for patients who have had a surgical or percutaneous intervention. One form will be completed for each intervention performed prior to enrollment.

Tissue Collection Form (Appendix K)

This form will be completed by research staff to record information related to the collection of any tissue that will be sent to the repository. Most of the tissue collected will be from the aorta but other types, such as skin, muscle and fat, may also be collected.

Completed forms will be keyed into a secure web-based data entry system accessible through the project website. RTI will monitor the promptness of data entry and quality of reported data on an ongoing basis, working closely with GCC research staff to address any problems with data collection.

Imaging Studies to Imaging Core

In addition to completion of the hard copy data forms described above, coordinators will send imaging studies of interest from study subjects to the Imaging Core at Medstar Research Institute. Unless otherwise specified in the signed consent and approved by the GCC IRB, imaging studies will be de-identified. Images will be sent either on a CD by an overnight courier, such as Federal Express, or by uploading to a secure website. Imaging studies of interest include the most recent Echo, MRI and CT at the time of enrollment, studies done at the time of

surgery or other event, and studies done during the interim period between enrollment and follow-up. The data obtained from the images and transmitted to RTI will be similar to the data requested on the Imaging Evaluation Form.

5.2 Follow-Up Data

Patients in the Registry will be followed for the life of the study according to their inclusion diagnosis, with the Loeys-Dietz patients being followed most frequently because of their rapid disease progression. The GenTAC Steering Committee has agreed to the follow-up intervals listed in the table below.

Condition	Follow-up Interval
Marfan syndrome	2 years
Turner syndrome	2 years
Ehlers-Danlos syndrome	2 years
Loeys-Dietz syndrome	1 year
TGFBR1 and TGFBR2 mutations	1 year
FBN1 mutation	2 years
Bicuspid aortic valve	2 years
Familial Thoracic Aortic Aneurysm and Dissections	2 years
Shprintzen-Goldberg syndrome	2 years
Other aneurysms and dissections of the thoracic aorta not due to trauma.	2 years

As part of the follow-up visit to the clinical center, several data collection forms will be completed, as appropriate, including:

- Clinic Evaluation Form
- Imaging Evaluation Form (when the image is not available to send to the Imaging Core)
- Non-Thoracic Imaging Evaluation
- Genetics Form
- Surgical and Percutaneous Interventions Form

Clinic Evaluation Form (Appendix L)

This form will be completed by research staff for patients at the time of follow-up. It will include a subset of items from the enrollment form, and collect information on conditions and health related events that have occurred since enrollment.

Imaging and Non-Thoracic Imaging Evaluation Forms (Appendices G and H)

These forms will be completed by research staff to record relevant information from the most recent echo-cardiogram, CT scan or MRI when the image is not available for transport to the Imaging Core. Additional forms may be completed for other imaging evaluations that occurred since enrollment.

Genetics Form (Appendix I)

This form will be completed by research staff to record any genetic testing that has been obtained since enrollment.

Surgical and Percutaneous Interventions Form (Appendix J)

This form will be completed by research staff for patients who have had a surgical or percutaneous intervention since enrollment. One form will be completed for each intervention.

Completed forms will be keyed into a secure web-based data entry system accessible through the project website. RTI will monitor the promptness of data entry and quality of reported data on an ongoing basis, working closely with GCC research staff to address any problems with data collection.

In addition to completion of hard copy data forms, coordinators will send imaging studies obtained since enrollment to the Imaging Core at Medstar Research Institute. As noted above, unless otherwise specified in the signed consent and approved by the GCC IRB, imaging studies will be de-identified. Images will be sent either on a CD by a courier, such as Federal Express, or by uploading to a secure website.

Follow-up of Vital Status

The consent form includes a request for permission to send a subject's social security number or name and birthdate to the National Death Index (NDI) if the subject cannot be reached after two years of attempted contact. The report from NDI will be reviewed by the GCC, and if a match is determined (i.e., the subject is deceased) the GCC will complete the Off Study Form.

5.3 Biological Specimens

Blood Collection

A peripheral venous blood sample will be obtained from each consenting participant during study enrollment. Whole blood specimens will be collected by trained nurses or phlebotomists into pre-labeled evacuated (i.e., Becton Dickinson [BD] Vacutainer or Greiner Vacuette) tubes. The amount of blood and tubes collected will depend on the age of the patient.

- For children less than 5 years of age, a total of 6 ml will be collected in one 6 ml ACD Solution A tube (Greiner - Vacuette). The blood will be used for cryopreservation of viable lymphocytes and establishment of a cell line.
- For children 5-12 years of age, 11 ml of whole blood will be collected in a 6 ml ACD tube Solution A tube (Greiner - Vacuette) and a 5 ml plasma preparation tube (PPT – BD Vacutainer) that contains sprayed dried K_2EDTA and a polymer gel barrier. The blood will be used for plasma biomarkers, DNA and cell lines.
- For patients at least 13 years of age, 18.5 ml of whole blood will be drawn in one 8.5 ml ACD Solution A tube and two 5 ml BD PPTs. The blood will be used for plasma biomarkers, DNA and cell lines.

Immediately following collection, each tube will be thoroughly mixed and then stored in an upright position at the appropriate temperature (i.e., room temperature, refrigerated or wet ice, to be determine) until it can be centrifuged (see below). The ACD tubes will be shipped at ambient temperature overnight to the RTI repository for processing. The PPTs will be centrifuged locally and the plasma transferred to a cryovial and frozen on the day of collection (within two hours of collection).

All whole blood specimens will be handled following CDC universal blood and body fluid collection guidelines. Collection procedures will follow OSHA blood borne pathogens standards and study specific operating procedures to prevent exposure to blood-borne pathogens and maintain specimen integrity.

Buccal Cell Collection

Subjects who are reluctant or cannot provide a blood sample for the collection of genomic DNA will be offered an alternative non-invasive procedure. To date, non-invasive alternative methods have focused on the collection of saliva samples that contain oral epithelial cells (buccal cells) and white blood cells. Collection procedures will be performed by using either swabs, brushes, mouthwash rinses or by expelling untreated saliva into a collection cup or tube that is mixed with a liquid preservative (i.e., DNA Genotek - Oragene).

Excess Tissue Collection

Some patients will undergo surgical intervention as part of their clinical care. The Registry will request that excess tissue be sent to the NHLBI Repository and become part of the biospecimen inventory available to study investigators. Tissue types of interest include aorta, heart valve, skin, skeletal, muscle and fat.

Specimen Identification

All biospecimen collection tubes will be identified with a preprinted subject ID label and a 2D matrix sample ID barcode label that also contains human readable text. The 2D sample labels will be generated using BSI-II, the NHLBI Web-based biospecimen management and inventory system that stores repository and study specific specimen data. A consecutive range of 4000 sample IDs will be reserved in BSI for exclusive use by the GenTAC Registry for blood and saliva samples.

Each collected tube and processed specimen vial will be labeled with a unique BSI ID number that links a study and collection date to a specific subject and specimen type. The BSI ID consists of a seven-character alpha numeric sample ID and a four digit vial ID in a specified format and structure (e.g. XX 99999 - 0001). The seven character sample ID provides a direct link to a specific subject, collection date and study while the four digit vial ID links a specific specimen to an individual sample ID.

The labeling of tissue samples will be done as needed with a separate set of specimen IDs. A unique number will be assigned to each tissue specimen based on the date of collection. A consecutive range of 1000 sample IDs will be reserved in BSI for exclusive use for all tissue samples.

Process, Inventory and Storage of Specimens

All whole blood and saliva specimens will be processed at the clinical site (GCC) or the RTI Repository following standardized and approved protocols. At a minimum, whole blood specimens collected in K_2EDTA PPT tubes will be fractionated by centrifugation at the GCC. After centrifugation, the plasma, will be aseptically transferred and dispensed into labeled cryovials and the vials stored at a minimum of -70 degrees C until they are transferred to the RTI Repository. Saliva specimens will be collected and then minimally processed (based on collection method) and shipped to the RTI Repository for inventory and storage.

The ACD tubes will be shipped at ambient temperature overnight from the GCC to the RTI Repository. The ACD whole blood samples will be inventoried and then separated by density gradient and centrifugation to obtain purified lymphocytes. The purified cells will be washed, counted and stained to determine pre freeze viability. The purified cells will then be combined with a cryoprotectant solution, aliquoted into cryovials at the appropriate concentration and then frozen in a programmable rate control freezer until the samples are cooled to -100 degrees C. The frozen samples will then be rapidly transferred to liquid nitrogen vapor phase storage (minimum temperature -135 degrees C).

Removed surgical tissue will be divided into 3 pieces and treated immediately as follows. One piece will be snap-frozen in liquid nitrogen and the remaining two pieces will be stored in RNA Later and fixed in formalin, respectively.

Specimen aliquots processed at RTI will be entered for inventory and tracking directly into RTI's inventory system, BSI-II. This is the same system used by the NHLBI repository and will allow easy requisition and transfer of vials at any time. Vials will be stored according to their material type in either a -80 degree Centigrade freezer (plasma and cell pellets) or a vapor phase LN2 freezer (cryopreserved lymphocytes).

Processed specimen aliquots at the GCC will be recorded into an electronic file using a user friendly web-based Study and Specimen Inventory Management System (SSIMS) accessed through the GenTAC website. SSIMS allows the user to catalogue information on processed samples including dates of collection and processing, sample and vial ID numbers, material type, volume or concentration, viability, or any other specimen characteristic that may be helpful to identify, locate and track a specific sample vial. After cataloging, all processed vials will be placed into an identified -80 degree Centigrade freezer for storage. The specimen vials will remain at low temperature storage except for tissue in formalin until they are packed on dry ice for shipment to the repository. Formalin-fixed tissue will be stored at 4 C until shipment or it is embedded in paraffin. Fixed tissue should be embedded in paraffin as soon as possible after fixing in formalin.

Packing and Shipping Specimens

Depending on the material type, specimens will be shipped to the RTI repository. Prior to specimen shipment, the GCC will send an electronic message to RTI requesting permission to ship specimens. The notification request will include an electronic shipping manifest that identifies the study, site location, approved courier service (e.g., FedEx), anticipated date of shipment and a detailed listing of each specimen vial. The GCC will use SIMMS to identify the specimens and create the electronic manifest. Once permission is granted, the site will withdraw the specimens from freezer storage according to approved study protocols.

Specimen vials will be withdrawn from freezer storage. All vials will be packaged following the International Air Transport Association (IATA) Dangerous Goods Regulations (DGR) triple packaging method (1) a leak proof primary receptacle, (2) a leak proof secondary packaging with a sufficient quantity of absorbent material to absorb the entire contents of all primary receptacles, and (3) an outer packaging of adequate strength for its capacity, mass and intended use. All packed vials will be placed in insulated fiberboard containers and completely covered with a sufficient amount of dry ice to maintain specimen temperature at – 70C for at least 36 to 48 hours. All shipments will be packaged by trained study site personnel following the IATA DGR. Following courier pick-up, site personnel will notify RTI repository that the shipment has been accepted for transit. The notification will include the name of the courier service, date of shipment, assigned airway bill number, estimated date of delivery and an updated version of the electronic manifest.

Long Term Storage

Samples will be sent to the NHLBI central biospecimen repository contractor at the end of the study and , will be stored indefinitely or until the sample is used up. This storage procedure is stated explicitly in the patient consent. Patients may request in writing at any time that their samples be destroyed.

Testing

Samples sent to the RTI will be withdrawn for various types of testing, as funding permits. DNA will be extracted from cell pellets or from the cell line to allow for genetic

sequencing of candidate genes. Plasma may be tested for biomarkers that may be related to the diagnoses, outcomes, or response to treatments.

Return of Test Results to Study Subjects

Findings from this study may or may not have clinical implications to research participants. There is no plan to return individual test results to patients or their physicians. However, if testing on study samples is done as part of the conduct of the Registry, and the result determines a study subject or their family is at risk for a disease or complication, the GCC PI will be informed and encouraged to discuss the results with the patient. Every enrolled patient has the right to request test results determined on their study samples by submitting a written request to the PI at their GCC. Testing that is done by an outside investigator as part of an approved ancillary study will not be considered part of the patient's study record and will not be sent to the DCC or be made available to study subjects.

5.4 GCC Training and Certification

In order to ensure consistent and standardized data collection across the 8 GCCs, RTI will develop and implement a training program for the research coordinators based on the training manual and manual of operations. This training will review the detailed instructions on subject enrollment, study procedures, data collection, data management procedures, and procedures for specimen collection, processing and shipping. Coordinators will also be certified in the requirements for packaging biospecimens and offering them for transport by air according to the International Air Transport Association (IATA) and the U.S. Code of Federal Regulations (CFR).

Various scenarios will be utilized to evaluate each site coordinator's understanding of:

- Purpose of study
- Enrollment criteria (inclusion/exclusion)
- Strategies for outreach and enrollment
- Successful completion and editing of hardcopy forms
  - Successful use of Question by Question specifications
  - Recitation of key data elements on all forms
- Review and understanding of the
  - data entry system
  - management system
- Privacy and data security policies
- Demonstrated ability to collect family history and pedigree data
- Demonstrated ability to correctly abstract information from medical records
- Biospecimen collection, processing, packing and shipping within regulations governed by CFR Title 49 and the IATA Dangerous Goods Regulations (DGR)
- Successful completion of course for packaging and shipping dangerous goods
- Willingness to communicate with the DCC
- Willingness to promptly complete and enter data forms
- Willingness to meet target enrollment numbers

Successful completion of the scenarios will contribute to the certification procedures for the site. Certification is necessary in order to begin or continue data collection, and will be repeated if there are changes to the protocol or data collection procedures. DCC staff will make at least annual site visits to each GCC to review the procedures and certification requirements.

Clinical Data Collection

Clinical measures for GenTAC will most often be obtained by abstraction of various medical records, including GCC-specific pathology, imaging, and laboratory reports. The DCC

will train the GCC site coordinators to identify the source of information and record the values for each of the data collection items on all of the GenTAC forms. Abstracted data for the GenTAC Registry will be keyed directly into a web-based data entry program developed by RTI and accessed through the secure project website. RTI will monitor the promptness of data entry and data quality on an ongoing basis and relay these observations to the sites. Remedial action, such as additional training, will be provided for GCC staff that does not meet expected standards. In extreme instances, the GenTAC Steering Committee may decide that a GCC that is not performing as expected be replaced.

Biospecimen Collection, Processing and Shipping

In additional to training on data collection procedures, the RTI Biospecimen Manager will train the GCC staff responsible for biospecimen collection, processing, and transport in standardized procedures. GCC personnel responsible for packaging and shipping of the biospecimens to the NHLBI Repository will be trained in the CFR Title 49 and the IATA Dangerous Goods Regulations. After the first training session, additional training will be available as needed, either to supplement the initial training, train new personnel, or retrain staff members showing difficulties with assignments. Once the specimen collection and processing plan is finalized, RTI will document the steps clearly and concisely by creating a Specimen Chart that graphically presents the specimen plan from collection through storage, including labeling, quantities and storage distribution. This chart, accompanied by standardized protocols, will form the basis for the biospecimen procedures manual.

Site Visits

The DCC will make routine site auditing visits to each GCC to monitor adherence to protocol procedures on site. More frequent site visits will be made as necessary to provide training to new staff, retraining of existing staff in new or old procedures, or to meet with staff regarding how to improve performance matrices.

6.0 Data Management and Quality Assurance

There are various potential problems and complexities in handling data from multiple sites. Data management and quality control systems will be implemented to ensure the timely receipt of all clinic data and resolutions of data discrepancies and duplications.

6.1 Data Management System

Data will be entered into a password protected, secure web-based data management (DMS) system which meets the requirements for Level-2 security (see Section 10.0). Within this system, RTI will build in edit, range and validity checks on the data as they are being entered. In addition to data entry, the DMS will allow GCC staff to produce data management reports to monitor their own performance. The DCC will train site staff in data collection and management in accordance with the protocol and, specifically, in the use of the DMS.

In order to monitor enrollment, data flow, delinquent data, and data quality, the DCC project managers will run reports that monitor the performance of the individual GCCs. These reports will also be distributed periodically to the GCCs and NHLBI. The reports will show the number of patients enrolled, the number and type of forms submitted through the DMS, the number of incomplete and delinquent forms, and the number of unresolved data edits. The DCC will collaborate with the GCC staff to design a report that is most helpful in monitoring the conduct of the study and producing high quality data for analysis.

In addition to producing monitoring reports at regular intervals, the DMS will allow the quick generation of customized reports, such as requests for frequencies and cross tabulations of outcomes and patient characteristics.

6.2 Editing

Quality control checks will be programmed into the web-based entry system developed for collection of data from GCCs. Checks for internal consistency with respect to dates, acceptable ranges, required items, and skip patterns will be set up as validation at the time of data entry. In batch, the data will undergo additional automated, electronic edits that could not efficiently be included in the data entry screens, such as cross-form editing.

Edit checks that are performed in real time will be replicated by the DCC in batch. In addition, complex within-form and across-form consistency and logic checks will be applied at the DCC. These checks will be based upon the specifications appropriate to each of the data collection forms. Any failures will be reported to the GCC enrolling the patient electronically as error resolution reports. GCC personnel will enter the corrections for the keyed data into the electronic file, and an audit trail of corrections will therefore be maintained. The audit trail will be transmitted to the DCC with the keyed database. The error resolution will be done online via the data entry system or externally, using an MS Excel or MS Access database file format to protect the data at the level required by the GenTAC contract.[1]

6.3 Monitoring Reports

In order to monitor enrollment, data flow, delinquent data, and data quality, reports will be generated at least quarterly from the accumulating database at the DCC and distributed to GCC staff and the Steering Committee. Semi-annual summary reports will be sent to the OSMB. These reports will show the number of patients enrolled by condition at each GCC, as well as the number and type of forms received by the DCC, the number of delinquent forms, the number and type of biologic samples sent to the repository, and the number of unresolved data edits. The DCC will collaborate with the research coordinators and investigators to design reports that are meaningful and assist in monitoring the conduct of the study and producing high quality data for analysis.

6.4 Duplicate Enrollments and Pedigrees

Duplicate enrollments across the GCCs are possible and will be identified by the DCC through a computer algorithm that includes date of birth and diagnosis. The DCC will verify duplicate enrollments with the GCCs involved, and a decision will be made as to what the permanent subject ID will be for the follow-up visits of those patients.

Family history of disease and pedigree data will be obtained from the Family History of Disease Form completed by the study subject (or guardian) and reviewed by the research coordinator at the GCC. Family members enrolled in the Registry at different sites will be identified through a series of events including,

- Patient consent for communication between GCC study personnel about the patient's enrollment in the Registry
- Identification of duplicate pedigrees by the DCC using a computer algorithm and the first name, State of residence for affected family members

[1] Note - The only consideration when planning this type of edit system is the need to store the data outside the protective firewall so that it could be accessed at time of edit. In some instances, extreme confidentiality concerns may override the efficiency of such a system.

- Communication cards given to patients for distribution to their relatives. These cards/brochures will also be used to encourage eligible relatives to enroll in the Registry.
- Verification between GCC coordinators that study subjects are related

The Registry is aware that family members are considered secondary research subjects when private health and identifiable information about them is collected from primary study subjects (Botkin, 2001; Resnik and Sharp, 2006). Waiver of informed consent and protection of family members from research risks is discussed in Section 9.0.

6.5 Quality Assurance

Quality assurance will be maintained by the following processes, procedures and steps:

- Development of a standard protocol, data collection instruments and manual of operations;
- Development and implementation of an appropriate data management system that has undergone code review, unit testing and integration testing;
- Training and certification of GCC staff;
- Data entry screens that replicate the hard copy data collection forms;
- Data entry editing followed by more comprehensive editing of data at the DCC;
- A streamlined approach for correction of errors in data and audit trails to document data change transactions;
- Monitoring of timely entry of data by GCC staff and timely resolutions of data discrepancies;
- Regular conference calls between the DCC project managers and the GCC study coordinators to review progress and to address questions and concerns;
- Routine random selection of keyed records for 100% verification
- Periodic visits to GCCs by the DCC and other study staff to review operations; and
- Final check on data quality and completeness as part of the process of making final data files for analysis. In addition to the interactive data edits done during the data entry process, computer programs will be developed to check that all forms and samples have been received for each participant, and that the data are consistent across the various forms.

7.0 Statistical Analysis

The GenTAC Registry cohort will consist of 3,800 patients who received treatment for conditions related to genetically-induced thoracic aortic aneurysms and dissections. In general the analyses of the Registry data will be descriptive and exploratory in nature and are for the purpose of hypothesis-generating rather than hypothesis testing. With a sample size of 3800, there is sufficient power to detect differences among treatment or outcome groups.

The Registry research questions can be summarized into the following categories: 1) Diagnostic criteria and description of the population, 2) Morbidity and natural history outcomes related to diagnosis and severity of disease; 3) Phenotypic/anatomic predictors for selection of surgery, type of surgery, and surgical outcome; 4) Drug treatment and disease progression, and 5) Gene, molecular and proteomic markers on phenotypic variation and prognostics. Additional new research questions may be developed as the study progresses.

7.1 Primary Outcomes

Outcomes for the Registry include aortic aneurysm presence, location and size; dissection or rupture of aneurysm; need for surgery and type of surgery; success and complications associated with surgery; type of drug treatment; response to drug treatment (decrease in aneurysm size, number and rupture); death; hospitalization; quality of life variables (physical, emotional, pain etc); morbidity conditions such as myocardial infarction and stroke; history of diseases; and blood chemistry values.

7.2 Approach to Analysis

Patients enrolled in the Registry will be characterized by their demographics (e.g. age, gender), clinical conditions, treatment procedures, and self-reported health status. Categorical or ordinal variables such as conditions and treatments will be summarized by frequency distribution. Continuous variables such as aneurysm size and blood chemistry results will be summarized by mean, median, standard deviation, minimum and maximum value. Outliers and possible data errors will be detected for further formal statistical analysis.

Regression methods will be used to examine the relationship between specific factors and an outcome of interest while controlling for potential confounding factors. According to the nature or measurement of outcome variables, linear regression, logistic regression, multinomial logistic regression, or time-dependent covariate analysis, such as Cox proportional hazards will be performed. Random effects models will also be used because the Registry is conducted through multiple clinical centers. Outcome variables such as intervention and clinical diagnosis may vary with physician or surgeon's experience. Ignoring this effect in the regression analysis may result in underestimated standard errors of parameter estimates.

There is a possibility that we may have some missing data, and missing data can seriously affect the results. Ignoring missing data, or assuming that excluding missing data is sufficient, will increase the risk of reaching invalid and insignificant results. Also, missing data may reduce the precision of calculated statistics because there is less information than originally planned. Another concern is that the assumptions behind many statistical procedures are based on complete cases, and missing values can complicate the theory required. We will review mean, standard deviation, frequencies, number of missing and non-missing values, number of extreme values for all variables, and will examine data from several different angles using different diagnostic reports to understand the missing data. We will conduct missing value analysis to find if the cases with missing values are systematically different from cases without missing values. Based on our initial missing value analysis we will employ appropriate ad-hoc imputation methods (such as, last observation carried forward, minimum value replacement, maximum value replacement, estimates based on different regressing models) or multiple imputations to impute the missing values.

7.3 Statistical Methods

Statistical analysis of the Registry data will require a variety of methods. Different statistical techniques will be employed to answer specific research questions. The selection of statistical methods will be based on the research question and the type of outcome variables used (categorical or continuous). The following are a few examples of statistical methods we will use to explore the specific type of scientific relationship between variables.

Descriptive and Bivariate Analyses

Descriptive analysis will be performed to examine the distribution of data and detect possible error. To explore the crude association of an outcome variable with a single factor we will use bivariate analysis. Selection of statistical methods will depend on the measurements of

two variables. Cross-tabulation will be used when both variables are categorical. Chi-square, or Fisher's exact test in the case of sparse cells, will be performed to test the significance of the associations such as the relationship between surgical procedures or surgical outcomes and the inclusion diagnoses (Marfan syndrome, etc). T-test and analysis of variance (ANOVA) will be used to compare the continuous outcomes such as absolute size or rate of change in the dimension of the aortic annulus among different outcomes. Simple correlation coefficient will be used to describe the association between two continuous or interval variables such as age disease severity.

Regression Analysis

To examine the relationship between a continuous outcome of interest, such as size of aortic dilation, and a group of factors that may be significantly associated with that outcome variable, multiple linear regressions will be used. Multiple regression will also be used to test the relationship between outcome variables while controlling for potential confounding factors. Logistic regression will be used to analyze binary outcomes, such as whether or not an individual patient was given a surgical intervention and whether or not the patient survived during a short period after surgery. Logistic regression will be used to analyze those binary outcomes in relation to predictors such as phenotype-specific indicators for surgery, risk factors for a short term survival status, anatomic predictors for dissection, aneurysm, and response to therapy, and predictors that affect the diagnosis of a specific condition. Multinomial logistic regression will be used to analyze categorical outcomes such as different surgical intervention procedures, and different aortic involvement (e.g., aneurysm, dissection, repair).

Time to Event Analysis

To analyze the survival type data, also known as time to event(s) data, we will use Kaplan-Meier survival analysis. To examine the relative risk for specific factors while adjusting for other covariates and potential confounding factors we will use Cox-proportional hazard model. If the proportional hazard assumption isn't valid for the Cox model, we will use Exponential regression and Weibull regression. If the survival or failure data have multiple failure events then we will use Multiple decrement life table analysis to calculate the cumulative incidence rate.

Other advance statistical analysis methods such as Mixed models, GEE, competing risk survival model, factor analysis, principal component analysis, and discriminant analysis may be also employed to answer scientific questions utilizing the Registry data. Analyses will mainly be implemented using SAS and other statistical package such as R, Stata, and MLwin.

8.0 Data Security

DCC (RTI International). Hard copy files will be stored in a locked filing cabinet with access only to authorized study personnel. A comprehensive set of IT security measures will be applied to all data collection and processing systems. These controls are appropriate for the Level 2 security described in the DHHS Matrix of Minimum Security Safeguards (i.e., NIST Moderate). These measures include a combination of physical security controls, operational controls, technical controls and management controls. All security controls are discussed in detail in the project specific IT Systems Security Plan (ISSP).

Briefly, there is a project website with a secure, gated data entry application. The data entry application requires the use of two factor authentication. Access is restricted and only authorized users are allowed entry into specific areas and are granted certain functional privileges. Access is controlled via security roles which are administered by RTI. Only members of certain security roles have view and/or edit permissions within the website.

Transmission of data keyed through the access restricted area is secure using *Secure Socket Layer* (SSL) technology. SSL is a security protocol that has become the universal standard for authenticating Web sites to Web users, and for encrypting communications between users and Web servers. SSL allows users to send and receive information in an encrypted manner as it travels through the many computers and networks before reaching its final destination. Because SSL is built into all major browsers and Web servers, simply installing a digital certificate, or Server ID, enables SSL capabilities.

Additional standard data security procedures will include daily incremental and weekly full backups of keyed data. These general procedures allow for daily copying of changed data sets to tape and weekly backups of the entire data archive. All backup tapes are stored in an offsite location.

GenTAC Clinical Centers (GCCs). All hard copy study records and the link between the patient name and the subject ID will be kept at the GCCs in locked filing cabinets with access limited to authorized study personnel. Each GCC has a unique login and password to enter the secure web-based application for data entry.

9.0 Human Subjects Protection and Informed Consent

No data collection activities will begin at an individual GCC until approvals from NHLBI, RTI, and the GCC-specific IRBs have been granted. The GCCs and RTI will concurrently submit the protocol, consents, and data collection forms to their respective IRBs for review. Upon approval, the signed documentation will be sent to NHLBI with a request to begin patient enrollment. No contacts with potential study subjects at an GCC will take place prior to NHLBI approval to commence enrollment. All participating GCCs and RTI have a Federal Wide Assurance issued through the US Office of Human Research Protections which assures that the organizations are complying with all Federal regulations to protect research subjects.

Retention, Protection, and Use of Identifying Information

Full names and other identifying information, excluding date of birth, will be retained only by the GCCs. Participants' data and biologic samples will be labeled with coded identification numbers that can be linked to names only by the corresponding GCC. GCCs may contact patients every 6 months to collect current contact information, which will assist in retention for follow-up. Personal identifying information, such as full name, address and social security number will not be provided to RTI or NHLBI. RTI will maintain a separate file of subject's first name and State of residence in order to identify possible duplicate or overlapping pedigrees and family members that are enrolled in the Registry.

Confidentiality of Data

All collected data will be kept confidential to the extent permitted by law. The study coordinator at each GenTAC Registry GCC will be trained in the requirement for maintaining confidentiality. In addition, a Certificate of Confidentiality to limit the possibility of forced disclosure under subpoena or other legal action will be requested through the NHLBI. The DCC will not be able to link an individual to their identifying information.

Family Members as Secondary Research Subjects

The Registry is cognizant of the concerns and limitations placed on obtaining identifiable health and private information about relatives from study subjects (Botkin, 2001; Resnik and Sharp, 2006). Waivers to informed consent will be requested from the IRB committees at the GCCs and DCC. Waivers should be granted if 1) the research involves no more than minimal risk, 2) the waiver will not adversely affect the rights and welfare of the family members, 3) the

research could not practicably be done without the waiver, and 4) when appropriate, the family members will be provided with additional pertinent information after participation. Strong data security systems will be in place to prevent any breach of privacy or confidentiality.

Parents of Minors as Primary Research Subjects

If the consenting parent of minor subjects will be completing the Family History Form, the parent will sign a consent form in order to provide information about their personal health. The child consent will serve as a combination parental permission and parent consent form.

Risks and Benefits

The data collected for this Registry are from medical record abstraction, patient interviews, blood collection and remnant tissue. The interviews are not considered greater than minimal risk but may trigger uncomfortable feelings about one's lifestyle, quality of life or personal or family history of disease. There are no direct benefits to the participants in this Registry. Some patients may benefit from knowing that they are helping to advance knowledge for future patients with their condition.

Access to Registry Data Results by Participants

Findings from this study may or may not have clinical implications to research participants. There is no plan to return individual test results to patients or their physicians. However, if testing on study samples determines a study subject or their family is at risk for a disease or complication, the GCC PI will be informed and encouraged to discuss the results with the patient. Every enrolled patient has the right to request test results determined on their study samples by submitting a written request to the PI at their GCC. Testing that is done by an outside investigator as part of an approved ancillary study will not be considered part of the patient's study record and will not be sent to the DCC or be made available to study subjects.

Unbiased Recruitment

All eligible participants will be recruited without bias. Genetically induced thoracic aortic aneurysms and related cardiovascular conditions under study are known to occur in all genders, races and age group. Children, women and minorities will be included as much as possible in the GenTAC Registry. Each GCC has provided a breakdown of targeted patients by age and gender in Table 1 on page 15. Breakdowns by race and Hispanic origin were not available, but will be monitored closely during the enrollment phase.

Inclusion of Children

Children will be included in the GenTAC Registry to the extent that they exist in the population being studied and meet eligibility criteria set forth for study participation. Marfan, Loeys-Dietz. and Turner syndromes are usually diagnosed in childhood. In addition to parental/guardian consent, child assent will be obtained from children at least 8 years of age, or as determined by the local IRB, up to age 21. All patients under the age of 8 will be consented solely by their parent or guardian.

Inclusion of Women and Minorities

We recognize the importance of having all eligible persons participate in this study, and we are particularly conscious of the need to include women and minorities in the study population. The DCC will monitor each GCC to assure recruitment of women and minorities who meet eligibility criteria. This study's selection criteria include all individuals that fit the inclusion criteria, without regard to sex, race, or ethnic group. Aortic aneurysms are not known to have a significant sex or race bias in terms of disease prevalence, and the expected enrollment figures for each GCC reflect the demographics of the site population as a whole.

Rights of Refusal and Withdrawal

Patients will be free to refuse enrollment or withdraw from the study at any time. Participants may refuse to answer individual questions on the self-administered questionnaires or may refuse the questionnaires entirely. In the case of withdrawal, participants have the right to request that their biological specimens be destroyed.

10.0 Data Use and Dissemination

Sharing of research data expedites the translation of research results into knowledge and procedures to improve human health. Data from well-characterized population samples constitute an important scientific resource. It is the view of the NHLBI that their full value can only be realized if they are made available, under appropriate terms and conditions consistent with the informed consent provided by individual participants, in a timely manner to the largest possible number of qualified investigators. The definition of "timely release and sharing" is three years after the completion of the enrollment phase of the study. Ancillary studies conducted concurrently with the Registry must also abide by the Data Sharing Policy.

10.1 Data Sharing Policy

The primary goal of the GenTAC Registry is to establish a data and biospecimen inventory that will be shared with Registry investigators and qualified researchers outside the Registry interested in studying additional aspects of genetically induced thoracic aortic aneurysms that are not being addressed by this protocol. De-identified patient-level data and biological specimens will be made available to researchers outside the Registry through an application and approval process as part of the study's Ancillary Studies Policy and Data Dissemination Plan. To protect the confidentiality and privacy of the subjects, investigators granted access to the limited access data and biologic specimens must adhere to strict requirements incorporated into a standard Data Use Agreement. In accordance with NHLBI policy, outside researchers will also be required to submit an approval from their Institutional Review Board (IRB).

Information on the types and quantities of biological specimens available to investigators will be shared according to the “Guidelines for Human Tissue Repository,” in which NHLBI provides specific guidelines for applying, reviewing, and processing requests for biospecimens collected through the Institute’s contracts. These guidelines include Specimen Request for Performance of Collaborative or Ancillary Studies, Materials Transfer Agreements, and Restricted Use of Specimens. As noted in Section 5.3, testing that is done by an investigator outside the Registry as part of an approved ancillary study will not be considered part of the patient's GenTAC study record, and will not be shared with the study participants.

10.2 Ancillary Studies Policy

The GenTAC Ancillary Studies Committee will develop a policy for the submission, review and approval of ancillary studies, as well as the role of the Scientific Advisory Board in the review of ancillary study proposals. An ancillary study is one that proposes to use GenTAC’s existing data and specimens or to collect new data and derives support from funds other than the GenTAC contract . GenTAC investigators are encouraged to consider ancillary studies and to involve other investigators within their institutions in this process. Ancillary study proposals will also be considered from non-GenTAC investigators and institutions.

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