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Factors associated with a positive test for Fabry Disease among genetic relatives of an affected Proband

By

Olivia Panepinto

Degree to be awarded: Master of Public Health

Epidemiology

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Jennifer Gladys Mulle

Committee Chair

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By

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B.S., State University of New York at Geneseo, 2013

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An abstract of
A thesis submitted to the Faculty of the
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Abstract

Factors associated with a positive test for Fabry Disease among genetic relatives of an affected Proband

By Olivia Panepinto

Fabry Disease is an X-linked lysosomal storage disorder resulting from mutations in the α-galactosidase gene (*GLA*) encoding the α-galactosidase A enzyme. In those affected, glycolipids accumulate in cells causing irreversible tissue damage. Symptoms of Fabry disease described in previous studies include extremity pain, unexplained fever, diminished sweating, decreased exercise tolerance, angiokeratoma, cornea verticillata, neuropathic pain, gastrointestinal issues, proteinuria, cardiac problems, and hearing loss. However, these symptoms are non-specific and occur in non-Fabry related conditions. In order to gain a better understanding of the Fabry disease symptom profile, we investigated the associations between self-reported symptoms and Fabry disease in genetic relatives of an identified Fabry disease proband (N=565). Family members of affected Fabry disease cases completed questionnaires about demographics, disease family history, symptom profile, and attached molecular and/or genetic test results of their affected family member. We examined associations between self-reported symptoms and a positive Fabry disease test. Testing positive for Fabry disease was associated with self-reporting corneal changes/whorls (OR: 6.30, 95% CI: 1.79, 22.1). Males with a first degree relative with Fabry and pain in their hands/feet were at a six fold increased odds of testing positive (OR: 6.37, 95% CI: 2.55, 15.91). Additionally, males experiencing decreased sweating (OR: 5.05, 95% CI: 1.78, 14.32) or angiokeratomas (OR: 6.4, 95% CI 2.3, 17.82) were at an increased odds for testing positive. These findings suggest that patients presenting with corneal changes, pain in hands/feet, decreased sweating or angiokeratomas may be useful in helping clinicians prioritize testing of relatives for Fabry disease.

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Table of Contents

Background

Epidemiology

Fabry Disease is an X-linked lysosomal storage disorder resulting from mutations in the α-galactosidase gene (*GLA*) encoding the α-galactosidase A enzyme (Schiffmann, 2009). It is characterized by extremity pain, unexplained fever, diminished sweating (hypohidrosis), decreased exercise tolerance, vascular skin lesions (angiokeratoma), cornea verticillata, neuropathic pain, gastrointestinal symptoms (abdominal pain/food intolerance), proteinuria and hearing loss in childhood (Daitx et al., 2015; Schiffmann, 2009; Wang, Bodamer, Watson, Wilcox, & Diseases, 2011) (Schiffman 2009, Daitx 2015, Wang 2011). As the disease progresses into the 3rd-5th decade of life, patients often suffer from renal and cardiac deterioration as well as ischemic stroke. Irreversible tissue and organ damage are inevitable among Fabry cases and renal transplants are often necessary during the late stages of disease (Schiffmann, 2009).

The phenotype was first described by Johannes Fabry and William Anderson in 1898. In 1965, Hashimoto et al. found bodies of endothelial cells, smooth muscle cells, fibrocytes and perivascular cells of patients with Fabry Disease during an electron microscopy study (Hashimoto, Gross, & Lever, 1965). They were described as overcrowded lysosomes, and etiology was likely genetic. The same year a family with 4 affected members led Dempsey to conclude that the disorder was sex linked, always appearing in hemizygous males and occasionally in heterozygote females (Dempsey et al., 1965).

Incidence of Fabry in males is estimated to range from 1:1,250 to 1:117,000 with a higher incidence of the later-onset/variant phenotype (Laney et al., 2013). In a newborn screening study of 37,104 consecutive male neonates, incidence was approximately 1:3,100 (Spada et al., 2006). In a study in which 75 probands were identified and their family pedigree constructed, the frequency of disease in female family members was twice that of males and thus incidence in females is predicted to be about 1:20,000 (Laney & Fernhoff, 2008). Unfortunately, case ascertainment for Fabry is low, due to the non-specific symptoms (Zarate & Hopkin, 2008). There are clusters of cases in Nova Scotia, Canada and West Virginia, likely due to founder effects. (Laney et al., 2013; Zarate & Hopkin, 2008).

The average age for the onset of symptoms is 6-10 for males and 9 for females (Laney 2013). From data obtained from the Fabry Registry, the current life expectancy is 58.2 for Fabry males compared with 74.7 in the US population. Fabry females live to 75.4 on average compared with a life expectancy of 80.0 in US females (Waldek, Patel, Banikazemi, Lemay, & Lee, 2009). The risk of death increases at age 40 and 60 in males and females respectively (Waldek et al., 2009). Cardiovascular disease is the most common cause of death for both genders (Waldeck, 2009). Additionally, Fabry Disease patients make up 3-4% of adult male cryptogenic stroke and unexplained left ventricular or hypertrophic cardiomyopathy patients (Zarate & Hopkin, 2008). Out of the three most common medical events (cardiovascular, cerebrovascular, and renal), renal replacement therapy was the most common first event experienced by patients (Waldek et al., 2009). Conversely, among all end stage renal disease patients, the prevalence of Fabry Disease is between 0.2% and 1.2% (Zarate & Hopkin, 2008). Before the availability of lifesaving

renal transplants, the average lifespan for someone with Fabry was 41-42 years old (Waldek et al., 2009). Fabry disease patients are at increased risk of mortality from both cardiovascular disease and renal disease.

Sex Differences in Disease Presentation

Due to the X-linked pattern of inheritance, males often exhibit what is known as the "classic phenotype" although some women may also exhibit this phenotype. In a cohort study of 98 males with Fabry disease, 77% reported having neuropathic pain and most said this pain persisted for life (MacDermot, Holmes, & Miners, 2001b). This study also reported a high proportion of males with angiokeratomas (71%), proteinuria (84%) and sensorial hearing loss (78%) (MacDermot et al., 2001b). Many of the males in this cohort exhibited characteristics consistent with a poor quality of life as 80.9% said Fabry affected their attendance at school and 36.3% said their social activities were limited by pain (MacDermot et al., 2001b).

Females often present with milder or later onset symptoms and can be referred to as having a late-onset/variant phenotype due to residual α-galactosidase A enzyme activity. Because they present with symptoms, women carrying *GLA* mutations are not just carriers of the genetic mutation (Deegan et al., 2006; Gupta, Ries, Kotsopoulos, & Schiffmann, 2005; MacDermot, Holmes, & Miners, 2001a; Riera et al., 2015; Smid et al., 2015; Wang, Lelis, Mirocha, & Wilcox, 2007). In fact, approximately 70% of females with the *GLA* mutation exhibit some Fabry symptoms while other studies found this figure to be from 90-100% (Wang 2007). In a study using Fabry Outcome Survey Data, the most common clinical symptoms reported in women were neurological (77% of

patients), cardiac (59% of patients) and renal (40% of patients) (Deegan et al., 2006). Neurological signs occurred earliest at an average of 16 years of age followed by cardiac (33.5 years old) and renal (37.3 years old) (Deegan 2006). Males can also present with a variant phenotype depending on the type of mutation in their *GLA* gene.

Molecular Genetics

Fabry disease is the result of a loss of function mutation in the *GLA* gene (Zarate & Hopkin, 2008). The α-galactosidase A gene (*GLA*) is located on chr Xq22 and is 12 kb long with 7 exons. It encodes the enzyme Alpha-galactosidase A, a lysosomal hydrolase that degrades neutral glycosphingolipids with terminal alpha-galactosylmoieties (Daitx et al., 2015). The gene encodes a 429 amino acid precursor protein which is processed into a 370 amino acid glycoprotein (homodimer). There are over 700 reported variants in the GLA gene. Pathological mutations are more frequent at buried locations due to solvent accessibility as well as highly conserved locations (Riera et al., 2015). Many studies have tried to discern pathological from neutral mutations and sequence properties have proven better than structural properties at differentiating the mutations (Daitx et al., 2015). In addition, structural and sequence properties have been successful in separating the classic from late-onset/variant phenotype (Saito, Ohno, Sese, Sugawara, & Sakuraba, 2010).

Glycolipids, especially globotriosylceramide (Gb3) begin to accumulate in endothelium, kidney, heart and nervous cells in the presence of a pathological mutation (Daitx et al., 2015). The accumulation of GB3 in the aforementioned cell types causes irreversible tissue damage and the typical symptoms of Fabry Disease. Evidence of GB3

storage in tissue has been observed as early as the prenatal period (Vedder et al., 2006). 5-10% residual enzyme activity may be enough to prevent Gb3 buildup (Zarate & Hopkin, 2008). Plasma lysosomal Gb3 level have been successful in discerning classic patients from individuals without Fabry but could not distinguish individuals with uncertain, late-onset/variant, or no Fabry Disease (Smid et al., 2015). There is no correlation between the type of mutation and lysosomal Gb3 levels.

Diagnosis and Treatment

Prior to molecular genetic testing, Fabry was diagnosed by identifying people with clinical symptoms typical of the Fabry disease phenotype. This method was not very useful because once symptoms develop, irreversible tissue damage has already occurred (Zarate & Hopkin, 2008). Additionally, because the clinical signs are non-specific, many patients were sent home with no diagnosis. Fabry is often confused with lupus, multiple sclerosis, arthritis, nonspecific peripheral neuropathy, fibromyalgia, chronic fatigue syndrome and hypochondria. Dried blood spots at birth can be used for screening purposes but participation in screening varies by state (Daitx et al., 2015). Current methods for diagnosing males and females vary. Males are diagnosed by measuring enzyme activity in the peripheral white blood cells or fibroblasts. Activity below 20% is confirmation that the individual has the disease while levels between 20-35% are of suspicion (Schiffmann, 2009). There is pseudo deficiency allele, D313Y, that causes reduced plasma alpha-gal A and reduced leukocyte enzymatic activity, thus *GLA* gene sequencing is used for a final validation (Yasuda et al., 2003). Because of random X inactivation in females, enzyme levels are insufficient for diagnosis as false negative

rates are high (~40%) (Deegan et al., 2006; Riera et al., 2015). Women should have their DNA genetically sequenced for the *GLA* mutation. Genetic sequencing is expensive and time consuming, therefore additional diagnostics are in development for women. Biochemical properties such as Km, Vmax, and thermostability may be useful in diagnosis patients whose enzyme activity is in normal ranges (Daitx et al., 2015).

There is currently no cure for Fabry Disease, but there are some promising therapies available that may delay the progression of the disease significantly. Two types of Enzyme replacement therapies (ERT) have been on the market since 2001: Replagal (Agalside alfa) and Fabrazyme (Algaside beta). Shire Pharmaceuticals withdrew their application to the FDA for Replagal approval in the USA in 2012 but Fabrazyme has been available to patients in the USA since 2003. ERT is not equally effective in all stages of disease and should be administered as early as possible. A ten year outcome study has recently been published for the use of Fabrazyme in 52 classic Fabry patients (50 men, 2 women) (Germain et al., 2015). 81% of the adults did not have any severe clinical events and 94% of patients were alive, a significant improvement in comparison to individuals who did not receive ERT. Additionally, of the 19% with clinical events, stroke was the most common event in patients under 40 while renal issues were most common in those 40 and older. The most successful treatment effects were observed in younger patients who started treatment at a younger age before organ damage began (Germain et al., 2015; Tondel et al., 2013). Another study examined the effect of ERT in children and young adults and found that long term treatment with ERT can result in complete globotriaocylceramide clearance of mesangial and glomerular endothelial cells

among all dose regimens and dose dependent clearance of the renal podocyte inclusions (Tondel et al., 2013).

Caring for Fabry Disease patients requires the coordination of many disciplines and includes genetic testing, test interpretation, genetic counseling, symptom monitoring, and coordination of therapy (Laney et al., 2013). Guidelines for genetic counselors and other health care professionals has been established using the current Fabry Disease literature (Laney et al., 2013). One of the recommendations includes creating a detailed pedigree once a proband is identified and testing all at risk family members (Laney et al., 2013). On average, 5 family members are identified with a *GLA* mutations for every proband (Laney 2008). Many descriptive studies have examined the prevalence of symptoms in the Fabry population but none have examined symptom profiles using an appropriate control group and their relation to family history (Deegan et al., 2006; Guffon, 2003; Gupta et al., 2005; MacDermot et al., 2001a, 2001b; Pitz et al., 2015; van der Tol, Sminia, Hollak, & Biegstraaten, 2016; Vedder et al., 2007; Wang et al., 2007; Whybra, Wendrich, Ries, Gal, & Beck, 2001). This study aims to depict which symptom(s) will best predict an individual's chance of testing positive for Fabry Disease and if these associations differ between family history and/or gender.

Methods

Study Population and Data Collection

In this cross-sectional study of 565 individuals who have family members with Fabry Disease, study subjects were ascertained from The Fabry Family Testing Program. This program is a collaboration between The Emory Genetics Laboratories and The

American Association of Kidney Patients and aims to test family members of known Fabry Disease cases. Eligible study subjects were relatives of individuals with Fabry Disease who were tested for a mutation in the *GLA* gene from 2008-2015. After informed consent (IRB# 00082495) study subjects completed a survey instrument with questions about demographics, disease family history, self-reported symptoms and attached the molecular and/or genetic test results of their family member who was previously diagnosed with Fabry disease to the questionnaire. In the current study the data were de-identified and the institutional review board at Emory University marked the study as "exempt" from review.

Individuals who filled out at least one piece of information on the data requisition form and were tested for mutations in the *GLA* genes through the AAKP/EGL testing program were included in the study (n=657). Ninety two individuals were removed from analyses due to missing data for either the outcome of interest (Fabry disease Status) or the covariates age and family history of Fabry disease. Additionally, five individuals were excluded for invalid family history specifications and one individual was excluded because they could not have been affected by Fabry disease based on family history and X-linked patterns of inheritance. After exclusions, there were 565 available subjects for analysis.

The outcome of interest, Fabry disease status, was determined by analyses of saliva DNA collected using Oragene Saliva Collection kits. Specimens were stored at room temperature and shipped within five days of collection by overnight delivery to the Emory Genetics Laboratory for gene sequencing. For targeted sequencing, the regions of DNA surrounding the mutation were amplified by PCR and sequenced in both the

forward and reverse direction. Individuals with any type of mutation in their *GLA* gene were classified as testing positive for Fabry disease and individuals with no mutations were classified as being negative for Fabry disease.

Self-report symptom data was reported on the questionnaire (Appendix A). Possible symptoms included: pain in hands/feet, numbness/tingling, chronic fatigue, purplish-red rash (angiokeratoma), decreased sweating, heat and/or cold intolerance, gastrointestinal problems, tinnitus or hearing loss, proteinuria, kidney failure, cardiac problems, TIAs/strokes, depression/anxiety, corneal changes or whorls, no symptoms , or "other". The "other" category included white space to describe the symptoms(s) experienced. Study subjects were also asked to indicate their relationship to relative(s) with Fabry disease by choosing from a list. The list included: mother, father, sister, brother, aunt, uncle, maternal grandmother, maternal grandfather, paternal grandmother, paternal grandfather, first cousin, or other.

Data analyses

Data from the survey tool were entered into Microsoft Excel. All data were cleaned and analyzed using SAS statistical software, version 9.3 (SAS Institute, Inc. Cary, North Carolina). Possible errors in data entry were verified against the original paper questionnaires. The outcome, Fabry disease status, was coded 1 if the individual had any mutation in the *GLA* gene and 0 otherwise. These mutations included known pathogenic mutations, likely pathogenic mutations, variants of unknown significance and benign mutations. Each individual's Family history response was reviewed for the closest degree relative with the disease and was consequently categorized into first,

second, or third degree closest relative using definitions from *The Clinical Genetics Handbook* (Ruth Y Berini, 1987).

The characteristics of individuals who tested positive and negative for Fabry disease were summarized using chi square tests for categorical variables and 2 sample t-tests for continuous variables. Fisher's exact methods were used in instances of sparse data. Results were considered statistically significant at $p \leq 0.05$ except in instances of multiple testing. To adjust for multiple comparisons (n=14 tests) the significance level was adjusted using a Bonferroni corrected p-value of $p \leq .004$.

Generalized estimating equations (GEE) were used specified with an exchangeable correlation structure to estimate odds ratios and 95% confidence intervals for associations between self-reported symptoms and Fabry disease diagnosis. These equations were used to account for the possible correlation of responses among families thus individuals were grouped into clusters based on their family *GLA* mutation, which served as a proxy for family. Interaction between the symptom of interest and potential effect modifiers (family history of Fabry disease, age and sex) were examined using the generalized score test statistic and backwards elimination. Based on previous literature and biological plausibility, potential confounders considered included age, sex, and first degree relative with Fabry disease. For consistent comparison between models, age, sex, and first degree relative with Fabry disease were adjusted for in all models.

The potential bias due to symptom misclassification for one of the symptoms: heat and/or cold intolerance was assessed. An external validation study for assessing the sensitivity and specificity of self-reported heat/and or cold intolerance in diagnosing hand-arm vibration syndrome (HAVS) was used to assign values to the bias parameters.

A survey was developed to screen for temperature intolerance and was compared to a gold standard apparatus for assessing temperature intolerance. Sensitivity was 94% and specificity was 52% (Pool, 2009). The expected association between self-reporting heat and/or cold intolerance and testing positive for Fabry disease was calculated using the following equations:

	Observation		Expected Truth	
	Heat/cold intolerance	No Heat/cold intolerance	Heat/cold intolerance	No Heat/cold intolerance
Fabry Disease	a	b	A=a- D+total (1-SP_{D+})]/[SE_{D+} - (1-SP_{D+})],	Fabry Disease-A
No Fabry Disease	c	d	C=[c- D-total (1-SP_{D-})]/[SE_{D-} - (1-SP_{D-})],	No Fabry Disease-C

Where,

SP_{D+} = specificity in non-cases, SP_{D-}= specificity in cases, SE_{D-}= sensitivity in non-cases and SE_{D+}= sensitivity in cases

Results

Selected characteristics of participants are summarized in table 1. Individuals who tested positive for Fabry disease were on average five years younger and more likely to be female than those who tested negative. They were also more likely to have a first degree relative with Fabry disease and self-report the following symptoms: pain in their hands and/or feet, corneal changes or whorls, angiokeratomas, decreased sweating, and heat and/or cold intolerance. Overall, those testing positive were also more likely to experience a greater number of symptoms in comparison to their negative testing

counterparts. Remaining self-reported symptoms (depression/anxiety, kidney failure, proteinuria, TIAs/strokes, tinnitus or hearing loss, cardiac issues, chronic fatigue, numbness/tingling) were not different between those testing negative and positive.

The distribution of selected characteristics of individuals who tested positive for Fabry by gender are displayed in table 2. Females who tested positive have slightly more first degree relatives with Fabry (82.62% vs 87.02%) and were slightly older, although the difference in mean age was not statistically significant. In comparing symptoms reported by individuals with a positive test between the two genders, males were significantly more likely to report pain in their hands/feet, kidney failure, angiokeratoma and decreased sweating. In contrast, females were significantly more likely than males to report corneal changes or whorls. The number of symptoms experienced was distributed evenly among males and females.

Adjusted odds ratios for the associations between testing positive for Fabry disease and self-reported symptoms are shown in table 3. After adjusting for sex, gender, and age, the odds of testing positive for Fabry disease was 6.3 times higher in those who reported corneal changes/whorls relative to those who did not report corneal symptoms (OR: 6.3, 95% CI: 1.79, 22.1, corrected p-value: 0.05). An over two fold increase in odds of testing positive was seen in those reporting decreased sweating (OR: 2.11, 95% CI: 1.26, 3.54, corrected p-value: 0.07), cardiac problems (OR: 2.12, 95% CI: 1.23, 3.64, corrected p-value: 0.08) and proteinuria (OR: 2.14, 95% CI: 1.01, 4.5, corrected p-value: 0.70) relative to those who tested negative. Additionally, a similar significant increased odds of testing positive for Fabry disease was observed in those reporting angiokeratomas (OR: 2.26, 95% CI: 1.19, 4.3, corrected p-value: 0.14) and pain in hands/feet (OR 2.20,

95% CI: 1.35, 3.61, corrected p-value: 0.03) compared to those who tested negative. Those reporting heat and/or cold intolerance were at a lower odds of testing positive in comparison to the aforementioned symptoms but they were still 80% more likely to test positive compared to testing negative (OR: 1.80 CI: 1.19,2.71, corrected p-value:0.07).

Some associations between a self-reported symptom and Fabry disease status differed significantly by family history (figure 1). Numbness or tingling was positively associated with testing positive in those with a first degree relative with Fabry (OR: 1.77, 95% CI: 1.17,2.69, corrected p-value: 0.11) while it was inversely associated with testing positive in those with a 2nd or 3rd degree relative with Fabry (OR: 0.43, 95% CI: 0.18,1.03, corrected p-value: 0.16). The same pattern was observed in those reporting gastrointestinal symptoms.

There were also instances where associations differed by gender (figure 2). Self-report of decreased sweating (OR: 5.05, 95% CI: 1.78, 14.32, corrected p-value: 0.03) and angiokeratomas (OR: 6.40, 95% CI: 2.30, 17.82, corrected p-value: 0.006) were both associated with testing positive for Fabry disease in males although the wide confidence intervals reflect imprecision in the risk estimate.

Pain in the hands and/or feet as well as heat and/or cold intolerance differed significantly by gender and family history (figure 3). Males with a first degree relative with Fabry and pain in their hands or feet were at a six fold increased odds of testing positive (OR: 6.37, 95% CI: 2.55, 15.91, corrected p-value: < 0.0001). Similarly, these same individuals were also five more times as likely to test positive if they reported heat and/or cold intolerance in comparison to negative testing males with first degree relatives (OR: 5.03, 95% CI: 2.27, 11.15, corrected p-value <0. 0001).

We aimed to calculate a bias adjusted odds ratio for the association between heat/cold intolerance and Fabry disease diagnosis. Assuming the values assigned to the bias parameters are correct, the expected true odds ratio could not be calculated because negative cell frequencies were generated from the bias model (table 4).

Discussion

In this exploratory study we compared the prevalence of commonly reported symptoms of Fabry disease in individuals who tested positive for Fabry disease to those who tested negative. After adjusting for multiple testing, our results from this study suggest that self-reporting corneal changes is significantly associated testing positive for Fabry disease among family members of known Fabry cases. Additionally, reporting decreased sweating and angiokeratomas is positively associated with testing positive for Fabry in males but not among females. Finally, indication of pain in hands or feet or heat/cold intolerance is strongly associated with testing positive among males of first degree relatives with Fabry disease.

Many of the previous studies conducted are descriptive in nature and examine prevalence of selected symptoms among individuals who have already been diagnosed with Fabry disease. This study differs in that we examined the distribution of self-reported symptoms among those who tested positive for Fabry disease compared to those who tested negative for Fabry disease and thus have a comparison group to make associative conclusions. Nevertheless, the prevalence of symptoms among those that tested positive can be calculated among our sample (table 2). In a systematic review, the prevalence of cornea verticillata in Fabry patients was estimated to be 69% compared to 7.2% in our

sample (van der Tol et al., 2016). However, in an individual registry study using data from the first 1,765 patients reported to the Fabry Registry, ophthalmological symptoms were present in 11% of males and 12% of females (Eng et al., 2007). Although the prevalence of corneal changes in the current study is low in comparison to other descriptive studies the odds of presenting with corneal changes/whorls is 6.3 times higher in those positive for a *GLA* mutation compared to those without a mutation. This is a very large difference and physicians may suspect Fabry disease in patients who present with corneal whorls. From the UK AFD register cardiac problems were reported in 53% of female patients and 56% of male patients (MacDermot et al., 2001a, 2001b) but only reported by 13% of Fabry confirmed cases in our sample. The may be explained by the fact that our sample was on average 6 years younger than the patients in MacDermot et al and the risk of cardiac problems increase with age. MacDermot et al. also reported 71% of males with proteinuria in their study compared to 9.7% in ours. Data from the UK AFD register is physician reported after the individuals were diagnosed with Fabry while individuals in this study self-reported their symptoms before knowing their diagnosis. The aforementioned symptoms would be very difficult to diagnose on one's own. Also, individuals in the registers comprise of cases that are more severe (sought medical attention for symptoms) and thus may be an overestimate of the prevalence in the general Fabry population. This may explain the discrepancy between our prevalence estimates and those reported using register data.

In our study, 29.3% of female Fabry patients reported gastrointestinal symptoms. This is in agreement with estimates from previous studies (11%-90%) although it is notable that this range is very wide (Bouwman et al., 2012; Eng et al., 2007; Galanos et al., 2002;

Guffon, 2003; MacDermot et al., 2001b; Whybra et al., 2001). In a case-control survey of female Fabry patients, cases were 3.72 times more likely than controls to report decreased sweating (95% CI: 1.26, 11.0) compared to 1.24 more likely in our study (95% CI: 0.74,2.1).

This study is prospective: information on symptoms was collected before disease status was revealed and thus bias in reporting symptoms based on disease was minimized. In contrast with previous studies our study was comprised of a comparison group to make associative observations and our sample was large (N=565) (Bouwman et al., 2012). The previous descriptive studies had sample sizes of less than 100 (Bouwman et al., 2012; Galanos et al., 2002; Guffon, 2003; Gupta et al., 2005; Kobayashi, Ohashi, Sakuma, Ida, & Eto, 2008; MacDermot et al., 2001a, 2001b; Wang et al., 2007; Whybra et al., 2001). Van der tol. and colleagues conducted a systematic review with a total of 753 cases but only looked at one symptom.

A limitation of the current study is that all of the individuals tested had relatives with Fabry disease and were aware of the range of symptoms associated with the disease prior to filling out the questionnaire. This may have caused over reporting of symptoms. However, this reporting is likely non-differential with respect to Fabry disease status because the symptoms were recorded before they were tested for the disease. Because non-differential misclassification of an exposure biases toward the null our estimates are likely conservative. Additionally, the survey instrument used was not validated and it is possible that the wording of the symptoms descriptions was misinterpreted especially in relation to non-descript symptoms (ex. heat/cold intolerance, pain in hands/feet).

The potential bias due to symptom misclassification for one of the symptoms: heat and/or cold intolerance was assessed. A bias adjusted odds ratio could not be calculated because negative cell frequencies were obtained. However, it is important to note that the ability for people who truly do not have heat/and or cold intolerance to identify their symptom through self-report is about as good as flipping a coin (specificity = 52%). When performing a sensitivity analyses of the bias analyses we found that after holding sensitivity constant (0.94), that the threshold for specificity in which non-negative expected cell frequencies were obtained was 0.81. For a non-specific symptom such as heat/cold intolerance, a future data requisition form may include a more specific definition.

Also, in classifying people as positive or negative for Fabry, we did not discern between "pathogenic", "likely pathogenic" or "neutral" mutations due to the fact that many of the mutations identified were private family mutations. However, because the people in this study had family members who were diagnosed with Fabry disease it is likely that many of the *GLA* mutations identified are pathogenic. Additionally, this sample may not include individuals with severe manifestations of disease and is not generalizable to the overall Fabry disease population. Cases with severe symptoms would likely have been identified as cases before this study and thus would not be included in this sample.

Because our study relied on self-report for symptom classification it was concerning to us that about ten percent of our sample comprised of individuals below the age of three. These young children may not have been able to verbalize how they were feeling and parents who filled out the questionnaires would have had to rely heavily on diagnosis from the child's pediatrician or their observations. Fabry disease is a progressive disease so less severe symptoms are typically seen in the younger patients and the average

age of onset of symptoms is 6-10 and 9 for males and females respectively (Laney 2013). After removing children under the age of three from analyses, our estimates did not change vastly (Appendix B).

Professional recommendations by genetic counselors (Laney et al., 2013) should be followed for diagnosing suspected/possible Fabry disease patients. The results of this study may enhance those recommendations. Males with first degree relatives with Fabry that exhibit pain in their hands and/or feet or heat/cold intolerance and all males with decreased sweating or angiokeratomas should be considered as possible cases and be tested accordingly. Additionally, the same precaution should be taken in all individuals that present with corneal changes/whorls. Future studies may consider using a validated questionnaire for assessing symptoms in conjunction with medical records to gain a more accurate depiction of symptom prevalence.

Table 1. Selected characteristics of individuals who have family members with Fabry disease; from data collected by the Fabry Family Testing program.[a] (N=565).

Characteristic[b]	Fabry Disease (n=278)[c]	No Fabry Disease (n=287)[c]	P value[d]
Self-Reported Symptoms (%)			
Pain in hands/feet	35.3	23.3	< 0.001
Corneal changes/whorls	7.2	1.1	< 0.001
Depression/anxiety	18.0	22.3	0.2
Gastrointestinal problems	30.0	25.1	0.24
Kidney failure	1.4	0.7	0.44[e]
Proteinuria	5.8	5.2	0.78
TIAs/strokes	3.2	2.1	0.4
Tinnitus or hearing loss	15.1	16.7	0.6
Angiokeratoma (purplish-red rash)	9.7	5.2	0.04
Cardiac problems	12.6	8.7	0.13
Decreased sweating	19.1	12.2	0.02
Chronic fatigue	22.0	19.2	0.41
Heat and/or cold intolerance	24.8	17.4	0.03
Numbness/tingling	26.6	24.0	0.48
Number of Symptoms (%)			0.28
0	35.2	42.1	
1-3	38.1	35.9	
4-6	16.9	15.3	
7+	8.7	6.6	
Closest Degree of Relation (%)			0.01
First degree relative with FD	85.0	77.7	
Second degree relative with FD	11.5	15.0	
Third degree or higher relative with FD	2.5	7.3	
Age, y	28.6 (21.5)	33.7 (23.7)	0.01
Male (%)	25.2	40.1	< 0.001

Abbreviations: TIA, transient ischemic attack, FD, Fabry Disease

[a]Fabry disease defined as having a mutation in the GLA gene.

[b]Continuous variables presented as mean (SD) and categorical variables as percentages.

[p]ercentages may not add up to 100% due to rounding

[d]T-test for continuous variables and chi-square test for categorical variables unless otherwise indicated.

[e]Fisher's exact test used.

Table 2. Selected characteristics of individuals with family members who have Fabry disease who themselves also tested positive by gender; from data collected by the Fabry Family Testing program[a] (N=278).

Characteristic[b]	Males (n=70)[c]	Females (n=208)[c]	P value[d]
Self-Reported Symptoms (%)			
Pain in hands/feet	47.1	31.3	0.02
Corneal changes/whorls	1.4	9.1	0.03
Depression/anxiety	12.9	19.7	0.2
Gastrointestinal problems	30.0	29.3	0.91
Kidney failure	4.3	0.5	0.02
Proteinuria	7.1	5.3	0.56
TIAs/strokes	1.4	3.9	0.32
Tinnitus or hearing loss	15.7	14.9	0.87
Angiokeratoma (purplish-red rash)	17.1	7.2	0.02
Cardiac problems	12.9	12.5	0.94
Decreased sweating	32.9	14.4	<.001
Chronic fatigue	15.7	24.0	0.15
Heat and/or cold intolerance	31.4	22.6	0.14
Numbness/tingling	27.1	26.4	0.91
Number of Symptoms (%)			0.63
0	32.9	36.5	
1-3	35.7	38.9	
4-6	18.6	16.4	
7+	12.9	8.2	
Closest Degree of Relation (%)			0.49
First degree relative with FD	82.62	87.02	
Second degree relative with FD	12.9	11.1	
Third degree or higher relative with FD	4.3	1.9	
Age, y	25.33(20.63)	29.39(24.66)	0.13

Abbreviations: TIA, transient ischemic attack, FD, Fabry Disease

[a]Fabry disease defined as having a mutation in the GLA gene.
[b]Continuous variables presented as mean (SD) and categorical variables as percentages.
[p]ercentages may not add up to 100% due to rounding
[d]T-test for continuous variables and chi-square test for categorical variables unless otherwise indicated.

[e]Fisher's exact test used

Table 3. Adjusted associations between testing positive for Fabry disease[a] and self-reported symptoms in individuals who have a family member with Fabry disease; from data collected by the Fabry Family Testing Program (N=565).

Symptom	POR	Lower 95% CI	Upper 95% CI	P-value	Bonferroni Adjusted P-values
Corneal Changes/Whorls	6.29	1.79	22.10	0.004[b]	0.05
TIAs/Strokes	2.30	0.73	7.08	0.15	1.00
Kidney Failure	3.14	0.56	17.60	0.19	1.00
Chronic Fatigue	1.28	0.84	1.96	0.24	1.00
Depression/Anxiety	0.83	0.52	1.36	0.48	1.00
Cardiac Problems	2.12	1.23	3.64	0.006	0.08
Tinnitus or Hearing Loss	1.24	0.78	1.96	0.36	1.00
Angiokeratoma (purplish-red rash)	2.26	1.19	4.30	0.01	0.14
Decreased Sweating	2.11	1.26	3.54	0.005	0.07
Proteinuria	2.14	1.01	4.50	0.05	0.70
Numbness/Tingling	1.31	0.90	1.91	0.16	1.00
Gastrointestinal Problems	1.34	0.96	1.87	0.08	1.00
Pain in hands/feet	2.20	1.35	3.61	0.002[b]	0.03
Heat and/or Cold Intolerance	1.80	1.19	2.71	0.005	0.07

Abbreviations: POR, prevalence odds ratio, TIA, transient ischemic attack

[a]Fabry disease defined as having a mutation in the GLA gene.

[b]Significant after Bonferroni Correction

Table 4. Bias adjusted odds ratio for the association between self-reporting heat/cold intolerance and testing positive for Fabry disease.

	Observation		Expected Truth	
	Heat/cold intolerance	No Heat/cold intolerance	Heat/cold intolerance	No Heat/cold intolerance
Fabry Disease	69	209	-140.09	382.09
No Fabry Disease	50	237	-190.78	385.78

Observed POR:	1.56	Expected Truth POR:	can't calculate

Figure 1. Adjusted odds ratios for symptoms associated with Fabry Disease according to closest family member with Fabry Disease (N=565)

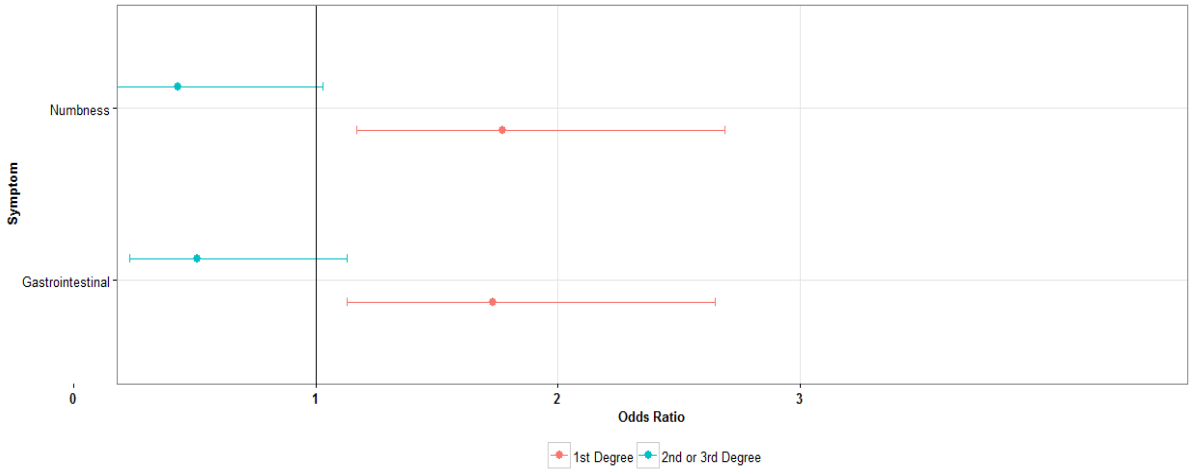


Figure 2.Adjusted odds ratios for symptoms associated with Fabry Disease according to gender (N=565).

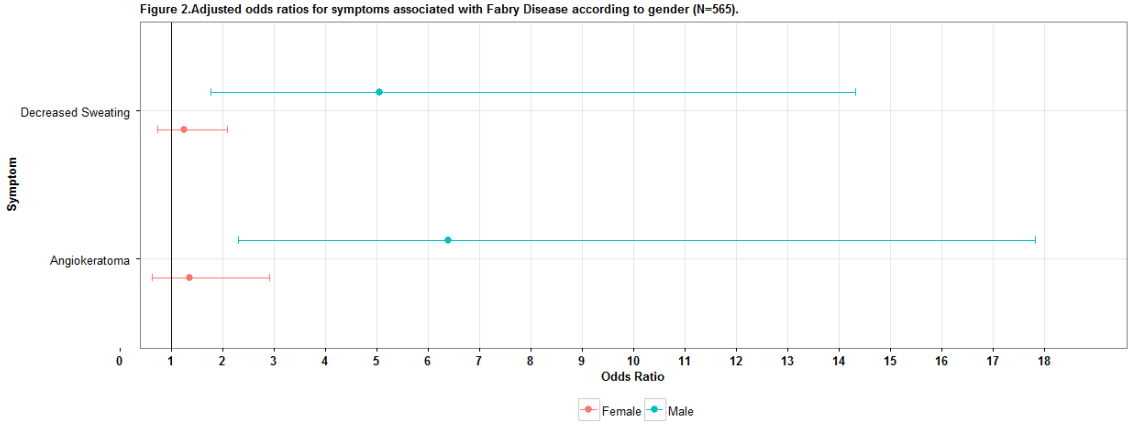
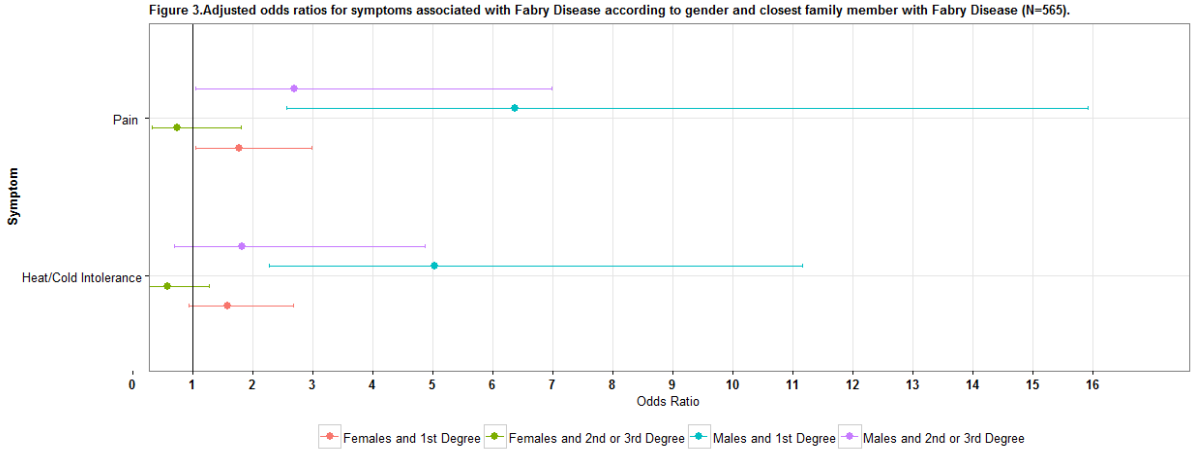


Figure 3.Adjusted odds ratios for symptoms associated with Fabry Disease according to gender and closest family member with Fabry Disease (N=565).



Appendix A

EMORY UNIVERSITY SCHOOL OF MEDICINE

Department of Human Genetics
Division of Medical Genetics
Lysosomal Storage Disease Center
http://www.genetics.emory.edu/LSDC/lsdc

aakp
American Association of Kidney Patients
www.aakp.org

FABRY FAMILY MEMBER REQUISITION FORM

I. History of Individual Being Tested for Fabry Disease: (check all that apply)

- ☐ Pain in hands/feet
- ☐ Numbness/tingling
- ☐ Chronic fatigue
- ☐ Purplish-red rash (Angiokeratoma)
- ☐ Decreased sweating
- ☐ Heat and/or Cold Intolerance
- ☐ Gastrointestinal Problems
- ☐ Other _____
- ☐ Tinnitis or Hearing Loss
- ☐ Proteinuria
- ☐ Kidney Failure
- ☐ Cardiac Problems
- ☐ TIAs/Strokes
- ☐ Depression/Anxiety
- ☐ Corneal Changes (Whorls)

II. Previous Testing:

Have YOU had any previous testing for Fabry disease?

- ☐ No prior testing/Unknown
- ☐ Enzyme analysis:
  - Date of testing: _____
  - Results: _____
  - Laboratory: _____
- ☐ Molecular/DNA analysis:
  - Date of testing: _____
  - Results: _____
  - Laboratory: _____

III. Patient's Family History:

Which of your family members are affected by Fabry disease? (check all that apply)

- ☐ Mother
- ☐ Father
- ☐ Brother
- ☐ Sister
- ☐ Niece
- ☐ Nephew
- ☐ Maternal Grandmother
- ☐ Maternal Grandfather
- ☐ Maternal Aunt
- ☐ Maternal Uncle
- ☐ Paternal Grandmother
- ☐ Paternal Grandfather
- ☐ Paternal Aunt
- ☐ Paternal Uncle
- ☐ Maternal cousin
- ☐ Paternal cousin
- ☐ Others _____

What is the name and relationship of the relative whose molecular test results including mutation are being sent with your sample?

Name: _____ Family Relationship: _____

Where and when was their molecular testing performed?

Date of testing: _____ Mutation: _____
Laboratory who performed the testing: _____

Appendix B

Comparison of adjusted associations between testing positive for Fabry disease[a] and self-reported symptoms among individuals of all ages (N=565) and in individuals age 4 and above (506).

	All Ages (N=565)				Age 4 and Above (N=506)			
Symptom	**POR**	**Lower 95% CI**	**Upper 95% CI**	**P-value**	**POR**	**Lower 95% CI**	**Upper 95% CI**	**P-value**
Corneal Changes	6.29	1.79	22.10	0.004[b]	6.06	1.72	21.33	0.01
TIAs/Strokes	2.30	0.73	7.08	0.15	2.30	0.72	7.41	0.16
Kidney Failure	3.14	0.56	17.60	0.19	3.25	0.56	18.98	0.19
Chronic Fatigue	1.28	0.84	1.96	0.24	1.25	0.82	1.91	0.31
Depression/Anxiety	0.83	0.52	1.36	0.48	0.83	0.52	1.34	0.45
Cardiac Problems	2.12	1.23	3.64	0.006	2.18	1.26	3.80	0.01
Tinnitus/Hearing Loss	1.24	0.78	1.96	0.36	1.22	0.77	1.94	0.39
Angiokeratoma	2.26	1.19	4.30	0.01	2.25	1.18	4.31	0.01
Decreased Sweating	2.11	1.26	3.54	0.005	2.01	1.20	3.54	0.005
Proteinuria	2.14	1.01	4.50	0.05	2.12	0.99	4.49	0.05
Numbness/Tingling	1.31	0.90	1.91	0.16	1.31	0.90	1.93	0.16
GI Problems	1.34	0.96	1.87	0.08	1.41	1.01	1.98	0.04
Pain in hands/feet	2.20	1.35	3.61	0.002[b]	2.22	1.33	3.70	0.002[b]
Heat and/or Cold Intolerance	1.80	1.19	2.71	0.005	1.76	1.16	2.68	0.01

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