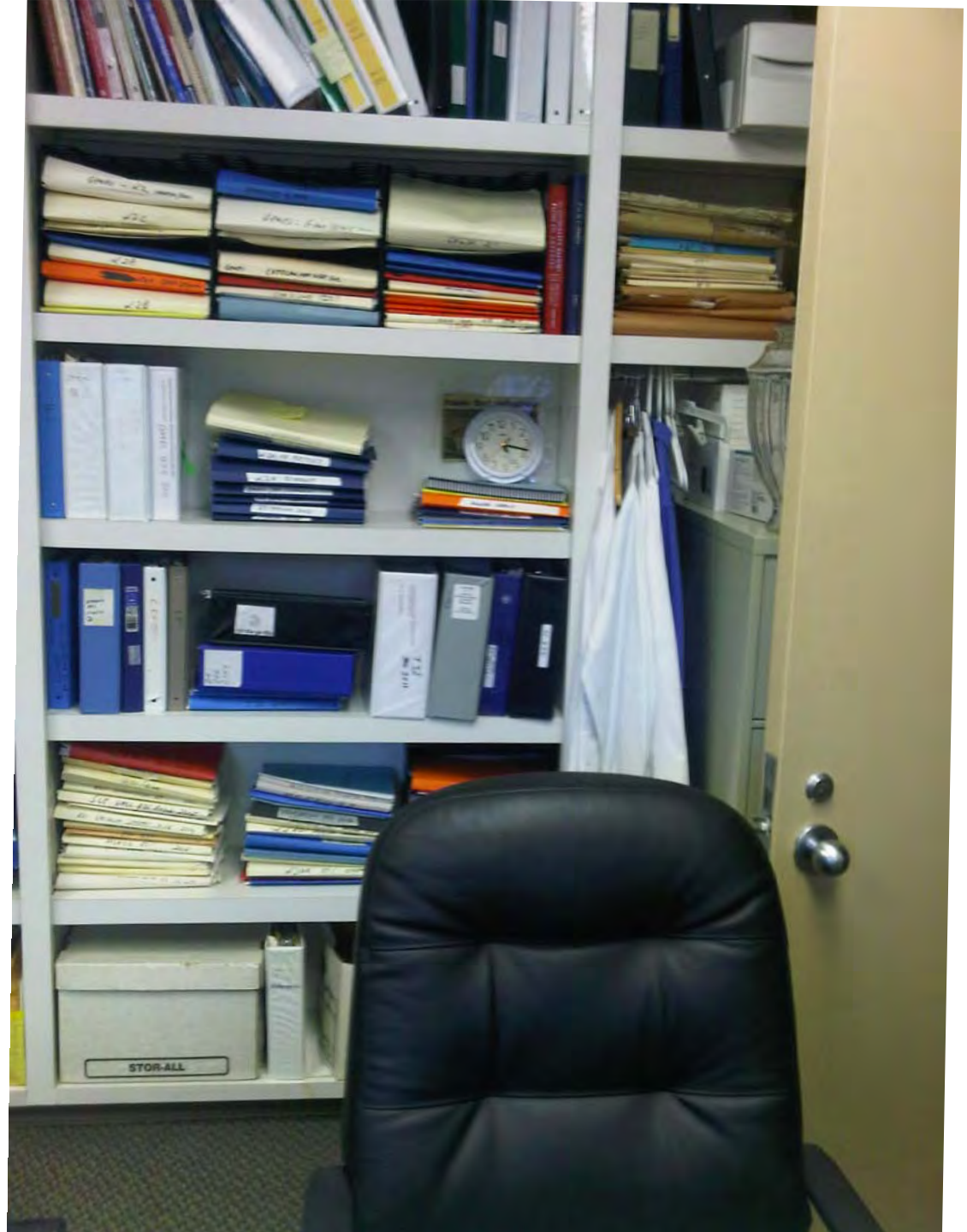

STOR-ALL

Writing the “Science” of a Grant

-blow their socks off, not your foot-

Overview

1. View the world as a reviewer
2. “The question”
3. Packaging “the question”
   Specific Aims, Abstract,
   Significance, Innovation, Approach
4. Common kisses of death

1. View the world as a reviewer

YOUR AUDIENCE: Reviewers are tired, overworked and grumpy speedreaders who are hungry for a reason to get rid of you

1. View the world as a reviewer

If your tired, overworked, grumpy, speed-reading reviewer finds one moderate weakness, he or she can pretty much stop reading. So, defensive writing.

Impact	Score	Descriptor	Additional Guidance on Strengths and Weaknesses
High Impact	1	Exceptional	Exceptionally strong with essentially no weaknesses
	2	Outstanding	Extremely strong with only negligible weaknesses
	3	Excellent	Very strong with only some minor weaknesses
Moderate Impact	4	Very Good	Strong but with numerous minor weaknesses
	5	Good	Strong but with at least one moderate weakness
	6	Satisfactory	Some strengths but also some moderate weaknesses
Low Impact	7	Fair	Some strengths but with at least one major weakness
	8	Marginal	A few strengths and a few major weaknesses
	9	Poor	Very few strengths and numerous major weaknesses

2. The Question

developing a good question takes time and a team

- It can take a year (or longer) to think though the questions underlying a grant
- Few grants fly on one person's expertise
- Start early. Writing is the wrapping up part
- Collaborate and get reviews (Specific Aims)

3. Packaging the Question

The most important parts of a grant are: 1) the Specific Aims and 2) the Abstract

Specific Aims

- Be specific; Be testable; Not be dependant
- Whole Specific Aims section less than a page;
- Less than 5 Aims (? 3 optimal); don't have too many subparts e.g. Specific Aim 1 d (iv)

SPECIFIC AIMS

Identify the knowledge gap?
What are you going to do to fix it ?

New and Interesting (Innovation)
Important (Significance)
Testable (Hypothesis)

A. SPECIFIC AIMS

Altered sympathetic responses are important in the pathogenesis and outcomes of a wide range of diseases. Genetic variation in adrenergic receptors (ARs) affects in vitro and in vivo responses. Little is known about the clinical importance of α_2ARs. The α_{2A}AR subtype, in addition to being the major effector of central inhibition of sympathetic activity, also mediates epinephrine-induced platelet aggregation and adrenergically-mediated suppression of insulin secretion. We and others have defined variability in the gene encoding the α_{2A}AR (*ADRA2A*) and characterized the in vivo consequences. We found that a common *ADRA2A* gain-of-function variant (haplotype 4) is associated with a greater response to agonist. Concordant with this finding, rs553668, a common *ADRA2A* variant that defines haplotype 4, is associated with increased epinephrine-induced platelet aggregation, and in a recent study in *Science*, with greater adrenergically-mediated suppression of insulin secretion, and increased risk of type 2 diabetes. Thus, several independent lines of evidence implicate *ADRA2A* variation, particularly rs553668 (haplotype 4), as a mediator of important differences in response, including differences in platelet aggregation and insulin secretion. Genetic variation in α_{2A}AR responses will be most important under conditions of adrenergic stimulation. There are no studies that address the clinical importance of *ADRA2A* variation under conditions of sympathetic stress. **Accordingly, in Project 3 we propose to test the overarching hypothesis that *ADRA2A* genetic variation is an important determinant of platelet aggregation and insulin secretion in pathological and physiological conditions that occur in the setting of adrenergic stimulation.**

Platelet aggregation in response to epinephrine is mediated by α_{2A}ARs. An increase in platelet aggregability concurrent with the early morning diurnal peak in sympathetic activity is well recognized, and is thought to account for the increased risk of myocardial infarction at this time of day. However, the contribution of genetic variability to this adrenergically-mediated diurnal platelet response is not known. Accordingly, we will define diurnal variation in epinephrine-induced platelet aggregation according to *ADRA2A* haplotype in healthy subjects studied under controlled conditions. **Specific Aim 1: Will test the hypothesis that *ADRA2A* haplotype affects diurnal platelet aggregation responses.**

Adrenergic stimulation, in addition to increasing platelet aggregation, also mediates inhibition of insulin secretion; this response is mediated by α_{2A}ARs and is affected by *ADRA2A* variation. A pathological situation where adrenergically-mediated regulation of insulin secretion is critical is stress-induced hyperglycemia. This occurs in 50% of patients with myocardial infarction and is associated with increased mortality. **Specific Aim 2: Will test the hypothesis that *ADRA2A* haplotype is associated with increased risk of stress-induced hyperglycemia in patients with myocardial infarction.**

Pregnancy is a physiological condition where increased sympathetic activation occurs in the setting of insulin resistance and a requirement for increased insulin secretion. Gestational diabetes - a condition occurring in 10% of pregnancies - occurs when the increased insulin resistance characteristic of pregnancy is accompanied by failure to secrete adequate insulin to maintain normoglycemia. The *ADRA2A* variant haplotype 4 (rs553668) results in greater adrenergically-mediated suppression of insulin secretion and is likely to increase the risk of gestational diabetes. **Specific Aim 3: Will test the hypothesis that *ADRA2A* haplotype is associated with increased risk of gestational diabetes.**

Aims 2 and 3 will be performed in BioVU, the de-identified Vanderbilt biorepository of DNA extracted from discarded blood collected during routine clinical testing, that can be linked to clinical and demographic data within the de-identified electronic medical record. These studies will therefore capture the genetic contribution to clinical conditions in real life clinical practice.

Specific Aims – Be Specific

Specific Aim 1: To collect information about SNPs and autonomic processes in hypertension

Specific Aim 2 : To correlate blood pressure with genetic changes

Specific Aim 3: To describe changes in biological processes that result from adrenergic genetic variation.

Specific Aim 4 : To create a mouse model that allows us to examine these processes

Specific Aims – Be Specific

Do not: collect, describe, collate, examine (unless an hypothesis), correlate, assess

Do: define, identify, test an hypothesis, determine mechanisms

Specific Aims – Do not shoot yourself in the foot

Specific Aim 1: To examine the hypothesis that flax seed juice is more effective than placebo juice in lowering blood pressure in a randomized, double blind, placebo controlled, parallel group 8 week study in 48 mildly hypertensive subjects.

Specific Aim 2 : To define the antihypertensive dose-response to flax seed juice by administering doses of xxxxxxx

Specific Aim 3: To determine the mechanism by which flax seed juice lowers blood pressure using 3 models that target the …..

The New 1+12 Page Application

- Significance
- Innovation
- Approach

Significance

Define the knowledge gap and its importance

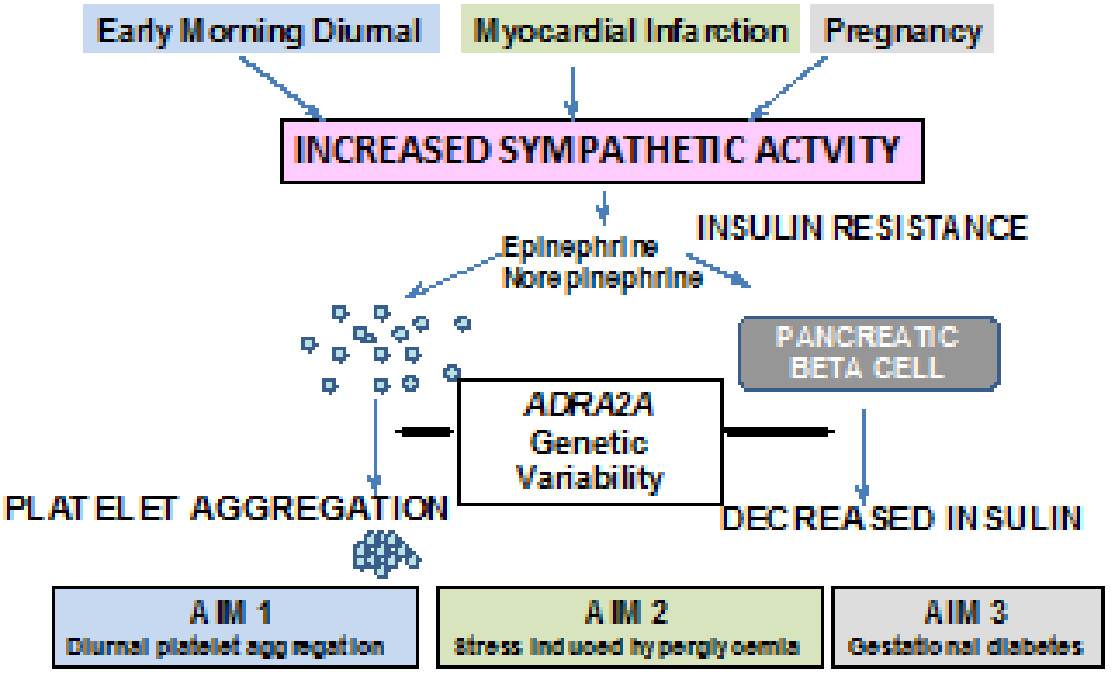
- Why want to do the study? Not a literature review
- Importance -Have to sell the idea (why should I care?)
- Need to convince the reviewer there is a knowledge gap and it matters
- If haven't hooked the reader here you are lost

Hypothesis/Specific Aim Figure

Summary of Significance of Proposed Studies

Our approach (See Hypothesis Figure below) will provide key insights into the mechanisms underlying interindividual differences in responses to sympathetic activation under physiological (diurnal variability, pregnancy) and pathological (myocardial infarction) conditions. The information derived from these mechanistically-defined phenotypes will have far reaching implications. For example, increased early morning platelet aggregation in a particular genotype would allow further studies to determine: if the risk of early morning myocardial infarction is selectively increased in this group; if diurnal resistance to antiplatelet therapy occurs; and if potentially selective antiplatelet therapy can be targeted to this subgroup. Similarly, stress-induced hyperglycemia and gestational diabetes are common; both are associated with adverse outcomes. Thus, identifying a genetic subgroup at increased risk will allow early identification and management. Additionally, there is the potential for developing α_{2A}AR agonists and antagonists that are selective for particular responses (e.g. insulin secretion).[8, 16] Thus, the proposed studies provide a logical, stepwise and targeted approach to translating the basic science of α_{2A}AR genetic variability to clinical significance, and have the potential to change our approach to a range of conditions including severe illness, pregnancy, and myocardial infarction.

Figure 4. SUMMARY OF HYPOTHESES



Innovation

- Challenges or shifts paradigms
- Advantages over existing methodologies
- Improvements or new applications of theoretical concepts or interventions
- Novel to more than one field

Example Frame for the Approach

- Rationale for Aim 1
- Approach
- Recruitment
- Inclusion Exclusion Criteria
- Study Protocol
- Methods
- Power Sample Size
- Statistical Analysis
- Anticipated Results
- Limitations, Pitfalls and Alternative Approaches

Approach

- Do it by Specific Aim
- Remind the Reader what the Aim is and why doing it (Rationale)
- Be very specific, reference your previous use of methods and tell them you have done this before.
- If you have methods common to Specific Aims e.g. SF36 then can either do in the first Aim where use or in a General Methods Section
- Cross reference clearly (see General Methods Section 2a, pg 18).

Approach

- Justify your choice of Methods
- Have a statistics section with power, sample size, analysis plan and a statistician
- Tell reader what you expect to find, what this means, and what unexpected problems or findings may occur.
- For K : tell reader how this will train you and where it will lead
- Claim ownership - what will you do
- Have a time line

Looks are important

- Try for 1 Fig or Table per page
- Leave some white space
- Short paragraphs
- Shortish sentences
- A bold heading for each paragraph that summarizes the paragraph

C. PRELIMINARY STUDIES

A long-term focus of our ongoing research is elucidation of the relationship between genetic variability and physiological and pharmacological response, particularly as regards explaining interindividual differences in cardiovascular response. We have performed many studies, several utilizing the same techniques we propose to use in the present proposal, that have contributed to our understanding of the relationship between phenotype and genotype.[56,7,11,57-70] In addition, Drs. Stein also participates in PharmGKB - an NIH funded consortium focused on SNP discovery and characterization. The focus of the Vanderbilt PharmGKB initiative is genes that modify arrhythmia, a focus that provides momentum and synergy to the present proposal since sympathetic activation is thought to play a role in the pathogenesis of sudden death.

The in vitro effects of the Arg16Gly and Gln27Glu β_2AR SNPs had been characterized, but little was known about their in vivo effects on vascular responses, particularly desensitization. This was important because one of the major in vitro phenotypes of these variants was altered desensitization. We therefore studied subjects selected to represent three common β_2AR haplotypes. Vascular responses were assessed by measuring changes in the diameter of a dorsal hand vein in response to continuous infusion of agonist – the same model we propose to use in Specific Aim 2. In vitro studies had suggested that the Glu27 variant was resistant to agonist-induced desensitization. We found that subjects who were homozygous for the Arg16 variant of the β_2AR had almost complete desensitization, the opposite of what would have been expected from the in vitro studies. Venodilation in response to isoproterenol in this group decreased from a mean of 44±11% to 8±4% (P=0.006).[61] This study showed for the first time in man that a common beta$_2$-AR polymorphism resulted in enhanced agonist-mediated desensitization in vivo, a finding with potentially profound implications regarding treatment with β_2AR agonists, as occurs in asthma, and also regarding the regulation of vascular response by β_2AR in other diseases such as heart failure. In addition to the more common β_2AR variants described above, there is an uncommon Thr164Ile polymorphism found in 0.5-2.3 % of individuals. This β_2AR variant has been associated with markedly altered responses to agonist in vitro; however, its effects on vascular responses in vivo had not been studied previously. We used the linear variable differential transformer dorsal hand vein technique to compare vasodilation in response to the βAR receptor agonist, isoproterenol, in healthy homozygous (Thr164/Thr164) (n=21) and heterozygous Thr164/Ile164 (n=5) subjects. The dose of isoproterenol required to achieve 50 percent venodilation (ED_{50}) (geometric mean; 95% CI) was markedly higher in subjects with the Ile164 allele (82.5ng/min; 17.3 – 394 ng/min) than those without (15.8 ng/min; 11-25 ng/min) (P=0.004)(Fig 4). Thus, the Ile polymorphism of the β_2AR, although rare, is important because it is associated with a 5-fold reduction in β_2AR vascular sensitivity. This finding suggests a mechanistic explanation for the clinical observation that survival was decreased in patients with congestive heart failure heterozygous for the Thr164Ile polymorphism.[72] These studies also illustrate several additional concepts relevant to the present proposal. First, it is important to perform experiments in vivo, since findings are often not what would have been predicted from the in vitro studies (e.g. ADRB2 Gln27Glu and desensitization) Second, although a variant may be relatively uncommon, even in the heterozygous form, if it has marked functional effects in vivo it may be of major clinical importance (e.g. ADRB2 Ile164). Third, our strategy of preselecting individuals with the genotypes of interest and studying them under closely controlled conditions in order to isolate the contribution of a particular genetic variant to response has been highly effective.[7,61] A common variant of ADRB1, an Arg389Gly variant, alters response in vitro. Initially, in order to address the question whether β_1AR variants affected response in vivo, we studied heart rate responses to graded exercise, a well-tested measure of β_1AR response, in subjects homozygous for the Arg389 and Gly389 variants, respectively. We,[67] and others,[73] found no effect of genotype, a surprising outcome considering the marked effects of this polymorphism in vitro. However, the increase in heart rate in response to exercise is affected by several factors in addition to β_1AR sensitivity, thus administration of a specific agonist would be a more direct way to test the hypothesis. Unfortunately, there is no specific β_1AR agonist available for use in humans, however, specific β_1AR antagonists are available. Therefore, resting and exercise hemodynamic responses were measured in subjects homozygous for Arg389 (n=21) or Gly389 (n=13) alleles before, and three hours after administration of a beta-blocker, atenolol. Genotype had a marked effect on resting hemodynamic responses to atenolol, with Arg389 homozygous subjects having a larger decrease in resting systolic (P < 0.001) (see Fig 5) and mean arterial (P = 0.009) blood pressure.[7] Thus, there is reduced sensitivity to a beta adrenergic receptor antagonist imparted by the Gly389 variant, and this polymorphism is an important determinant of variability in response to beta blockade. Our preliminary approach to studying the clinical consequences of ADRA2 genetic variants has been two-pronged. First, we have identified the variants present in the *ADRA2A*, *2B* and *2C* genes, since this was

NO

C. PRELIMINARY STUDIES

Genetic variation and cardiovascular response

A long-term focus of our ongoing research is elucidation of the relationship between genetic variability and physiological and pharmacological response, particularly as regards explaining interindividual differences in cardiovascular response. We have performed many studies, several utilizing the same techniques we propose to use in the present proposal, that have contributed to our understanding of the relationship between phenotype and genotype.[30,31,32,33] In addition, Drs. Stein also participates in PharmGKB - an NIH funded consortium focused on SNP discovery and characterization. The focus of the Vanderbilt PharmGKB initiative is genes that modify arrhythmia, a focus that provides momentum and synergy to the present proposal since sympathetic activation is thought to play a role in the pathogenesis of sudden death.

Beta$_2$ (ADRB2) adrenergic receptor genetic variants: functional effects

ADRB2 and desensitization: The in vitro effects of the Arg16Gly and Gln27 Glu β_2AR SNPs had been characterized, but little was known about their in vivo effects on vascular responses, particularly desensitization. This was important because one of the major in vitro phenotypes of these variants was altered desensitization. We therefore studied subjects selected to represent three common β_2AR haplotypes. Vascular responses were assessed by measuring changes in the diameter of a dorsal hand vein in response to continuous infusion of agonist – the same model we propose to use in Specific Aim 2. In vitro studies had suggested that the Glu27 variant was resistant to agonist-induced desensitization. We found that subjects who were homozygous for the Arg16 variant of the β_2AR had almost complete desensitization, the opposite of what would have been expected from the in vitro studies. Venodilation in response to isoproterenol in this group decreased from a mean of 44±11% to 8±4% (P=0.006).[34]

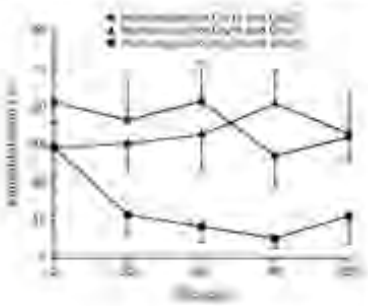
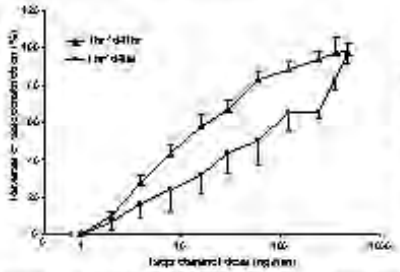


Figure 3 Vascular desensitization is markedly affected by β_2 adrenergic receptor haplotype.
From Dishy et al New Engl J Med 2001; 345:1030-1035[34]

This study showed for the first time in man that a common beta$_2$-AR polymorphism resulted in enhanced agonist-mediated desensitization in vivo, a finding with potentially profound implications regarding treatment with β_2AR agonists, as occurs in asthma, and also regarding the regulation of vascular response by β_2AR in other diseases such as heart failure.



ADRB2 and vascular response: In addition to the more common β_2AR variants described above, there is an uncommon Thr164Ile polymorphism found in 0.5-2.3 % of individuals. This β_2AR variant has been associated with markedly altered responses to agonist in vitro; however, its effects on vascular responses in vivo had not been studied previously.

Figure 4 The Ile allele of the Ile164Thr β_2 adrenoceptor variant is associated with a 5-fold reduction in vascular sensitivity to isoproterenol (P=0.004)[35]

We used the linear variable differential transformer dorsal hand vein technique to compare vasodilation in response to the βAR receptor agonist, isoproterenol, in healthy homozygous (Thr164/Thr164) (n=21) and heterozygous Thr164/Ile164 (n=5) subjects. The dose of isoproterenol required to achieve 50 percent venodilation (ED_{50}) (geometric mean; 95% CI) was markedly higher in subjects with the Ile164 allele (82.5ng/min; 17.3 – 394 ng/min) than those without (15.8 ng/min; 11-25 ng/min) (P=0.004)(Fig 4). Thus, the Ile polymorphism of the β_2AR, although rare, is important because it is

YES

A bold heading for each paragraph that summarizes the contents

Choosing phenotypes in which to study ADRA2A genetic variation

To define the clinical significance of ADRA2A genetic variation it is important to study phenotypes likely to be informative. Complex heterogeneous phenotypes such as hypertension and myocardial infarction are less likely to be informative than ones in which increased sympathetic activation is associated with a response of interest that is mediated at least in part by α_{2A}ARs. We have chosen 3 such situations: i) the increase in platelet aggregation in the early morning that is adrenergically-mediated and thought to contribute to the increased risk of myocardial infarction at this time of day; ii) stress-induced hyperglycemia in the setting of myocardial infarction; and iii) gestational diabetes.

Early morning platelet aggregation

We considered several potential phenotypes for studying α_{2A}AR mediated platelet aggregation under conditions of altered sympathetic activity (e.g. mental stress, exercise, an illness such as myocardial infarction, epinephrine infusion). However, we chose to study early morning platelet aggregation for the flowing reasons: it is a clinically important phenotype; altered α_{2A}AR responses have been implicated in its mechanisms; and studies can be performed in the absence of potent anti-platelet drugs such as are used to treat myocardial infarction. (note to myself - this may belong better in alternative app)

There is a marked diurnal fluctuation in the occurrence of myocardial infarction and sudden death, with

Kisses of Death

- Trivial question
- “We will thus confirm …”
- No story, doesn't hang together
- Intelligent but unintelligible

Kisses of Death

- Reader can't understand what you want to do
- Reader can't understand why you want to do it
  (fails the "So what" test)
- Invalid – Design, Analysis
- No power /sample size/statistician
- Sloppy

Kisses of Death for Science of a K

- the mentor is invisible
- the mentor is overpowering
- you are the mentor's lackey, no ownership, no path to independence; no new skills
- "if it works I am unclear where this will go" (show them the R on the horizon)

The Recipe

1. Plenty of gestation and preparation time
2. A new and important question
3. Write, write, write - every word, sentence and paragraph are important – details make the difference
4. Rewrite, rewrite, rewrite
5. Get reviews and listen to them
6. A thick skin

The Recipe (Preparation time 1 year)

1. Mix 2 cups of inspiration and 8 cups of perspiration and spread evenly on 13 pages
2. Cook for several months, stirring constantly
3. Taste and add much more perspiration
4. Serve with 2 cups of trepidation and 8 cups of composure
5. Repeat often till works

Resources

- Essential of Writing Biomedical Research Papers 2nd ed Mimi Zeiger
- Arnett DK Preparing Effective Grant Applications Circulation 2009;120:2607-12
- http://www.niaid.nih.gov/researchfunding/grant/cycle/pages/part05.aspx
- Internal Vanderbilt Resources
- Russell SW, Morrison DC. The Grant Application Writer's Workbook

Abstract

- Only thing most reviewers read
- Set “importance” stage
- Identify knowledge gap
- Therefore we propose to ….. in 3 Specific Aims. In Aim 1 we will test the hypothesis … Aim 2 …
- Close with some “significance”

Have a Time-Line Somewhere

(Career Development and Research Strategy) refer to it in text

Career Award Development Timeline					
	Year 1	Year 2	Year 3	Year 4	Year 5
Mentorship					
Weekly meetings	X	X	X	X	X
6 monthly Mentorship Committee	X	X	X	X	X
Didactic Coursework					
MSCI	X	X			
Writing Course 301 ABC			x		
Genetics 101 XYZ			X		
Genetics 201 XYA		X		X	
Annual Lukewarm Spring Meeting	X	X	X	X	X
Research					
Aim 1: To xxxxxxxx	X				
Aim 2: To xxxxx					
Obtain IRB	X	X			
Data analysis	X	X	X		
Writing MS			X	X	
Aim 3: To xxxxx					
Enrollment	X	X	X	X	
Genotyping and Laboratory Analyses			X	X	X
Data analysis and writing				X	X
R01 Preparation				X	X

- References
  - Primary source often but not always
  - Reasonable number, cite yourself, Refman
  - Style?
- Tables
  - Self Standing. Careful with Abbreviations
  - Data in Tables and text must match
- Figures
  - Reasonable number
  - Abbreviations
  - Quality
  - Measures of spread