BIOGRAPHICAL SKETCH

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NAME: Knowles, Joshua Wiley

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POSITION TITLE: Assistant Professor, Cardiovascular Medicine, Stanford University

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of North Carolina at Chapel Hill	BA	1991-1995	History, Biology
University of North Carolina at Chapel Hill	PhD	1997-2001	Genetics, Molecular Bio.
University of North Carolina at Chapel Hill	MD	1995-2003	Medicine
Stanford University	Residency	2003-2005	Medicine
Stanford University	Fellowship	2005-2010	Cardiovascular Medicine

A. Personal Statement

A major focus of my research has been to elucidate the genetic basis of insulin resistance (IR), which is a necessary but not sufficient precursor for T2D and a major cardiovascular risk factor. I started this work in conjunction with Dr. Gerald Reaven, the "father" of insulin resistance. Though Dr. Reaven unfortunately passed away earlier this year, we are continuing his pioneering work. It is estimated that 25-33% of the US population is sufficiently insulin resistant to have serious clinical consequences. Insulin sensitivity can be exquisitely quantitated using reference measures like the euglycemic clamp or insulin suppression test or estimated less precisely with surrogate measures like fasting glucose and insulin levels. **Unfortunately, therapeutic options for IR are very limited and there is an urgent need to identify new targets.**

I helped to lead an international effort to uncover IR susceptibility loci through the GENESIS (GENEticS of Insulin Sensitivity) project. GENESIS is a GWAS of ~2800 white individuals from 4 international cohorts with direct measures of IR (euglycemic clamp or insulin suppression test) plus four additional replication cohorts. We discovered a novel insulin susceptibility locus and showed that *in vitro* perturbations of *NAT2* caused marked effects on glucose uptake, lipolysis and adipogenesis. Furthermore, *Nat1* deficient mice have elevated fasting glucose and insulin as well as IR assessed by glucose and insulin tolerance tests (***Knowles** et al., JCI*, *2015;* **Chennamsetty** *et al. Cell Reports. 2016)*. Along with Dr. Ingelsson, we have used our GWAS data prioritize *FAM13A,* one of a cluster of "lipodystrophy-like" genes (***Yaghootkar*** et al., Diabetes 2014) representing a new, common paradigm linking adipogenesis, energy storage, ectopic fat deposition and IR. We have preliminary data showing that *FAM13A* deficiency improves glucose metabolism and other cardiometabolic parameters, which is consistent with human genetic data suggesting that excess *FAM13A* impair subcutaneous white adipose tissue deposition. Finally, I have also helped to lead an NIH-funded U01 effort to use iPSC technology to study IR. We created induced pluripotent stem cell (iPSC) lines on ~200 individuals we recruited for the scientific community to use as model systems for the study of IR and other conditions (**Carcamo-Orive** *et al*. *Cell Stem Cell*. 2017). These iPSC lines are being differentiated into tissues relevant to the pathology of IR (adipose, skeletal muscle and endothelial cells) and we have begun to map the key regulatory networks for IR and correlate this with the underlying genetic background of the individuals. Finally, we are working on a daily basis with data from the UK Biobank, exploring the associations of IR with multiple other phenotypes.

Clinically, I am cardiologist, lipidologist and attending physician in the Stanford Center for Inherited Cardiovascular Disease I direct the Familial Hypercholesterolemia (FH) clinic. I am also the Chief Medical

Advisor for the FH Foundation (www.thefhfoundation.org), a patient-founded and patient-led, non-profit organization for individuals with FH. FH is a hereditary condition caused by a variety of genetic mutations that lead to significantly elevated LDL cholesterol (LDL-C levels), contributing to a 20-fold increased lifetime risk for cardiovascular disease. Nevertheless, with proper statin-based treatment regimens morbidity and mortality approaches that of the general population. However, patients often have reservations about taking statins due to concerns about side effects. I was recently awarded a Doris Duke Clinical Investigator award to study the physiologic mechanisms of statin-associated diabetes.

1. Carcamo-Orive I, Hoffman GE, Cundiff P, Beckmann ND, D'Souza SL, **Knowles JW**, Patel A, Papatsenko D, Abbasi F, Reaven GM, Whalen S, Lee P, Shahbazi M, Henrion MY, Zhu K, Wang S, Roussos P, Schadt EE, Pandey G, Chang R, Quertermous T, Lemischka I. Analysis of Transcriptional Variability in a Large Human iPSC Library Reveals Genetic and Non-genetic Determinants of Heterogeneity. Cell Stem Cell. 2017 Apr 6;20(4):518-532.e9. doi: 10.1016/j.stem.2016.11.005. Epub 2016 Dec 22. PMID: 28017796, PMCID: PMC5384872
2. Chennamsetty I, Coronado M, Contrepois K, Keller MP, Carcamo-Orive I, Sandin J, Fajardo G, Whittle AJ, Fathzadeh M, Snyder M, Reaven G, Attie AD, Bernstein D, Quertemous T, **Knowles JW**. Nat1 deficiency is associated with mitochondrial dysfunction and exercise intolerance in mice. *Cell Rep*. 2016 Oct 4;17(2):527-540. PMCID: PMC5097870
3. **Knowles JW**, Xie W, Zhang Z, Chennemsetty I, Assimes TL, Paananen J, Hansson O, Pankow J, Goodarzi MO, Carcamo-Orive I, Morris A, Chen Y-DI, Mäkinen V-P, Ganna A, Guo X, Abbasi F, Greenawalt DM, Lum P, Molony C, Lind L, Lindgren C, Raffel LJ, Tsao PS, The RISC Consortium, The ULSAM Study, The EUGENE2 Study, The GUARDIAN Consortium, The SAPPHIRe Study, Schadt EE, Rotter JI, Sinaiko A, Reaven G, Yang X, Hsiung CA, Groop L, Cordell HJ, Laakso M, Hao K, Ingelsson E, Frayling TM, Weedon MN, Walker M, Quertermous T. Identification and validation of *NAT2* as an insulin sensitivity gene. *JCI. 2015;125(4):1739-51* PMCID 4409020.
4. Yaghootkar H, Scott RA, White CC, Zhang W, Speliotes E, Munroe PB, Ehret GB, Bis JC, Fox CS, Walker M, Borecki IB, **Knowles JW**, Yerges-Armstrong L, Ohlsson C, Perry JR, Chambers JC, Kooner JS, Franceschini N, Langenberg C, Hivert MF, Dastani Z, Richards JB, Semple RK, Frayling TM. Genetic evidence for a normal-weight "metabolically obese" phenotype linking insulin resistance, hypertension, coronary artery disease, and type 2 diabetes. Diabetes. 2014 Dec;63(12):4369-77. doi: 10.2337/db14-0318. Epub 2014 Jul 21. PMCID: PMC4392920

B. Positions and Honors

Positions and Employment

2003-2005	Medical Internship and Residency, Internal Medicine, Stanford University
2005-2010	Cardiovascular Medicine Fellow, Stanford University
2010-2014	Cardiovascular Medicine, Instructor
2012-current	Chief Medical Advisor, *The FH Foundation* (patient charity for Familial Hypercholesterolemia)
2014-current	Assistant Professor of Medicine (Cardiovascular Medicine), Stanford University
2017-current	Co-Director, Stanford Translational Investigator Pathway program
2017-2018	Associate Program Director, Stanford Cardiovascular Medicine Fellowship Program
2018-current	Program Director, Stanford Cardiovascular Medicine Fellowship Program

Professional Membership and Activities:

2012-	Fellow, American Heart Association
2012-	Diplomate, American Board of Clinical Lipidology
2012-	Fellow, American College of Cardiology
2013-	Organizing Committee and co-Host, annual international *Familial Hypercholesterolemia Summit* (2013 Annapolis, 2014 New York, 2015 Pasadena, 2016 Dallas, 2017 Miami)
2013	Writing Group, American Heart Association, *AHA Scientific Statement on Familial Hypercholesterolemia*. Chair of writing group: Sam Gidding
2013-2014	Member, ATVB Council & Lifestyle and Cardiometabolic Health
2013-2016	International advisory board: Canadian FH registry, led by Jacques Genest, McGill

Honors and Awards

1995	Graduated with Honors, double major in History and Biology, UNC
1996	John B. Graham Medical Student Research Society, UNC School of Medicine
2003-2004	Stanford Clinical Investigator Pathway
2005-2006	Stanford University School of Medicine Dean’s Fellowship

2006 The Future Leaders in CV Medical Research Program, Fellowship Award
2007, 2008 Edwin Alderman Excellence Award for Clinical Research, Stanford Cardiovascular Medicine
2015 ACCF/Herman K. Gold Young Investigator's Award Finalist in Molecular and Cellular Cardiology: "*Nat1* knockdown results in an insulin resistance phenotype in vitro and in vivo"
2016 Clinical Scientist Award, Doris Duke Charitable Trust

C. Contributions to science

The fundamental theme of my work is the use of genetics to understand and ameliorate human cardiovascular disease. This work spans the continuum of genetics from discovery of risk variants to the creation and use of in vitro and in vivo model systems for mechanistic studies to translation of genetic findings to the clinic to application of genetic information in public health efforts (in the context of Familial Hypercholesterolemia). I have ~ 90 publications including ~25 first, co-first or last author papers with an overall h-index of 33 and i10-index of 51 (Google scholar). My publications broadly relate to genetics and cardiovascular diseases with over 10 that focus specifically on insulin resistance (IR) and type 2 diabetes (T2D).

1. Defining the genetic basis of insulin sensitivity including creation of iPSC model systems.
Using the GWAS findings from the GENESIS consortium along with orthogonal data derived from multiple glycemic measurements we have been able to shed light on the classification of many T2D variants as either IR or pancreatic beta cell loci (*Dimas et al., 2013; Ingelsson et al*., 2010, referenced below).

Along with co-investigators Drs. Erik Ingelsson and Thomas Quertermous I am working on efforts to map the causal molecular mechanisms for IR-related T2D GWAS loci. We are using both informatics approaches, high throughput genetic studies (eg RNASeq) and in vitro and in vivo model systems to first define the causal genes at these associated loci and then to define the causal molecular mechanism.

For the last several years, I also helped to lead an NIH-funded U01 effort to use iPSC technology to study insulin resistance. We created induced pluripotent stem cell (iPSC) lines on ~200 individuals we recruited for the scientific community to use as model systems for the study of IR and other conditions. These iPSC lines are being differentiated into tissues relevant to the pathology of insulin resistance (adipose, skeletal muscle and endothelial cells). By studying the functional and transcriptional differences between cells derived from insulin resistant vs insulin sensitive individuals under basal and insulin-stimulated conditions we have begun to map the key regulatory networks for IR and correlate this with the underlying genetic background of the individuals. The iPSC lines and resultant genetic data have been deposited at WiCell and dbGaP respectively as a resource for the greater scientific community, our initial findings are under review at *Cell Stem Cell*.

1. **Knowles JW**, Assimes TL, Tsao PS, Natali A, Mari A, Quertermous T, Reaven GM, Abbasi F. Measurement of insulin-mediated glucose uptake: Direct comparison of the modified insulin suppression test and the euglycemic, hyperinsulinemic clamp. *Metabolism*. 2013;62:548-553. PMCID: 3925367
2. Ingelsson E, Langenberg C, Hivert MF, Prokopenko I, Lyssenko V, Dupuis J, Magi R, Sharp S, Jackson AU, Assimes TL, Shrader P, **Knowles JW**, Zethelius B, Abbasi FA, Bergman RN, Bergmann A, Berne C, Boehnke M, Bonnycastle LL, Bornstein SR…(> 20 authors)… Williams GH, Lind L, Barroso I, Quertermous T, Walker M, Wareham NJ, Meigs JB, McCarthy MI, Groop L, Watanabe RM, Florez JC. Detailed physiologic characterization reveals diverse mechanisms for novel genetic Loci regulating glucose and insulin metabolism in humans. *Diabetes*. 2010;59:1266-1275. PMCID: 2857908
3. Fall T, Xie W, Poon W, Yaghootkar H, Mägi R; GENESIS consortium, **Knowles JW**, Lyssenko V, Weedon M, Frayling TM, Ingelsson E. Using genetic variants to assess the relationship between circulating lipids and type 2 diabetes. Diabetes. 2015 May 6. pii: db141710. PMID: 25948681.
4. Dimas AS*, Lagou V*, Barker A*, **Knowles JW***, Magi R, Hivert MF, Benazzo A,…(>10 authors)… Collins FS, Mohlke KL, Tuomilehto J, Quertemous T, Lind L, Hansen T, Pedersen O, Walker M, Pfeiffer AF, Spranger J, Stumvoll M, Meigs JB, Wareham NJ, Kuusisto J, Laakso M, Langenberg C, Dupuis J, Watanabe RM, Florez JC, Ingelsson E, McCarthy MI, Prokopenko I. Impact of type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. *Diabetes*. 2013; PMCID: 4030103, * co-first author

2. Discovery of the genetic basis of complex cardiovascular disease. Through the use of high throughput genotyping and sequencing approaches the last decade has seen a revolution in the understanding of complex cardiovascular diseases. I helped to lead our GWAS of early onset coronary disease in the ADVANCE study and have collaborated with many other international consortia such as CARDIoGRAM, MAGIC and GIANT, for coronary heart disease, diabetes, and anthropomorphic traits respectively. We and others have begun to tease out the molecular mechanism of action of many of these loci and these results are now being translated clinically through the development of new therapeutics and risk prediction models.

1. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E, Saleheen D, Erdmann J, Goldstein BA, Stirrups K…(>3 authors)…**Knowles JW**…(> 20 authors)..O'Donnell C, Reilly MP, Marz W, Collins R, Kathiresan S, Hamsten A, Kooner JS, Thorsteinsdottir U, Danesh J, Palmer CN, Roberts R, Watkins H, Schunkert H, Samani NJ. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nature Genetics*. 2013;45:25-33. PMCID: 3679547
2. Kraja AT, Vaidya D, Pankow JS, Goodarzi MO, Assimes TL, Kullo IJ, Sovio U, Mathias RA, Sun YV, Franceschini N, Absher D, Li G, Zhang Q, Feitosa MF, Glazer NL, Haritunians T, Hartikainen AL, **Knowles JW**, North KE, Iribarren C, Kral B, Yanek L, O'Reilly PF, McCarthy MI, Jaquish C, Couper DJ, Chakravarti A, Psaty BM, Becker LC, Province MA, Boerwinkle E, Quertermous T, Palotie L, Jarvelin MR, Becker DM, Kardia SL, Rotter JI, Chen YD, Borecki IB. A bivariate genome-wide approach to metabolic syndrome: STAMPEED consortium. *Diabetes*. 2011; 60:1329-1339. PMCID: 3064107
3. Xie W, Wood AR, Lyssenko V, Weedon MN, **Knowles JW**, Alkayyali S, Assimes TL, Quertermous T, Abbasi F, Paananen J, Haring H, Hansen T, Pedersen O, Smith U, Laakso M, Dekker JM, Nolan JJ, Groop L, Ferrannini E, Adam KP, Gall WE, Frayling TM, Walker M. Genetic variants associated with glycine metabolism and their role in insulin sensitivity and type 2 diabetes. *Diabetes*. 2013;62:2141-2150. PMCID: 3661655

3. Improving the care of Familial Hypercholesterolemia (FH) patients. I am a cardiologist and I direct the Familial Hypercholesterolemia (FH) clinic at Stanford. I am also the Chief Medical Advisor for the FH Foundation (thefhfoundation.org), a patient-founded, non-profit organization for individuals with FH. FH is a hereditary condition caused by a variety of genetic mutations that lead to significantly elevated LDL cholesterol (LDL-C levels), contributing to a 20-fold increased lifetime risk for cardiovascular disease. Despite its high prevalence in the United States (1 in 200 individuals), it is estimated that less than 10% of FH patients are formally diagnosed. However, with proper family based screening, diagnosis and treatment, morbidity and mortality can be greatly ameliorated. I have lead and directed multiple efforts to improve the diagnosis and treatment of FH patients nationally and internationally including: Developing a national patient registry for FH; Participating in an AHA Scientific Statement on FH; Writing the *GeneReview* of FH; Writing an application to the Centers for Medicare & Medicaid Services to get specific ICD 10 codes FH (later approved).

With proper statin-based treatment regimens morbidity and mortality approaches that of the general population. However, patients often have reservations about taking statins due to concerns about side effects. We have shown that individuals with IR are particularly at risk and are orchestrating a trial to investigate the effects of statins on IR and insulin secretion (Doris Duke Foundation Clinical Investigator award).

1. **Knowles JW**, O'Brien EC, Greendale K, Wilemon K. Genest J, Sperling LS, Neal WA, Rader DJ, Khoury MJ.' Reducing the Burden of Disease and Death from Familial Hypercholesterolemia: A Call to Action. Am Heart J. 2014 Dec; 168(6):807-11. PMCID: PMC4683103
2. Youngblom E., **Knowles JW**. Familial Hypercholesterolemia. 2014 Jan 2. In: Pagon RA, Adam MP, Bird TD, et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2014.http://www.ncbi.nlm.nih.gov/books/NBK174884
3. deGoma EM, Ahmad ZS, O'Brien EC, Kindt I, Shrader P, Newman CB, Pokharel Y, Baum SJ, Hemphill LC, Hudgins LC, Ahmed CD, Gidding SS, Duffy D, Neal W, Wilemon K, Roe MT, Rader DJ, Ballantyne CM, Linton MF, Duell PB, Shapiro MD, Moriarty PM, **Knowles JW**. Treatment Gaps in Adults With Heterozygous Familial Hypercholesterolemia in the United States: Data From the CASCADE-FH Registry. Circ Cardiovasc Genet. 2016 Jun;9(3):240-9. Epub 2016 Mar 24. PMCID: PMC5315030

4. Using genetics to improve clinical care. My major focus has been the translation of genetic findings to the clinic. We have completed recruitment for a randomized clinical trial ("A Pilot Randomized Trial of Personal Genomics for Preventive Cardiology", ClinicalTrial.gov identifier NCT01406808) with a goal of determining whether patient outcomes are improved by providing patients information about their GWAS-verified coronary disease susceptibility variants. I am activity involved in efforts to understand the promise and limitations of whole genome sequencing efforts for clinical care.

1. **Knowles JW**, Assimes TL, Kiernan M, Pavlovic A, Goldstein BA, Yank V, McConnell MV, Absher D, Bustamante C, Ashley EA, Ioannidis JP. Randomized trial of personal genomics for preventive cardiology: Design and challenges. *Circulation. Cardiovascular genetics*. 2012;5:368-376. PMCID: 3394683
2. Ashley EA, Butte AJ, Wheeler MT, Chen R, Klein TE, Dewey FE, Dudley JT, Ormond KE, Pavlovic A, Morgan AA, Pushkarev D, Neff NF, Hudgins L, Gong L, Hodges LM, Berlin DS, Thorn CF, Sangkuhl K, Hebert JM, Woon M, Sagreiya H, Whaley R, **Knowles JW**, Chou MF, Thakuria JV, Rosenbaum AM,

Zaranek AW, Church GM, Greely HT, Quake SR, Altman RB. Clinical assessment incorporating a personal genome. *Lancet*. 2010;375:1525-1535. PMCID: 2937184

5. Evaluated the relationship between statins and diabetes. Statins increase the risk of diabetes, but the mechanism is unclear. Using several approaches including the analysis of clinical trial data as well as undertaking a trial (funded by the Doris Duke Foundation) where we are measuring insulin sensitivity and insulin production pre- and post- statin we are gaining insight into this phenomenon.

1. Kohli P, **Knowles JW***, Sarraju A, Waters DD, Reaven G. Metabolic Markers to Predict Incident Diabetes Mellitus in Statin-Treated Patients (From the Treating to New Targets (TNT) and the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) Trials). Amer. J. of Cardiology, 2016 Nov 1;118(9):1275-1281. PMID: 27614854
2. Ingelsson E, **Knowles JW**. Levering Human Genetics to Understand the Relation of LDL Cholesterol with Type 2 Diabetes. *Clinical Chemistry*. 17 May 2017. doi:10.1373/clinchem.2016.268565.
3. Reaven GM, **Knowles JW,** Leonard D, Barlow CE, Willis BL, Haskell WL, Maron DJ. Relationship between simple markers of insulin resistance and coronary artery calcification. *J Clin Lipidol*. 2017 Jun 6: pii: S1933-2874(17)30339-2. doi: 10.1016/j.jacl.2017.05.013.

Complete list of my publications from MyBibliography
http://www.ncbi.nlm.nih.gov/sites/myncbi/joshua.knowles.1/bibliography/10313470/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

NIH, R01DK107437 *(PI: Quertermous)* 4/01/16- 3/30/20
Molecular Mechanisms of IR Associated Loci
Brief Project Description: The goals of this work are to identify causal variation and causal genes in type 2 diabetes GWAS loci and to initiate studies to determine the mechanism of the association.
Role: Co-Investigator

Merck, LKR#151615 (PI: Quertermous) 1/01/16 – 12/31/18
Evaluation of FAM13A as an IR gene
The goals of this work are to determine the role of FAM13A on IR using in vitro and in vivo models.
Role: Co-Investigator

Doris Duke Charitable Trust, SPO#: 124038 (**PI: Knowles**) 7/01/16–6/30/19
Statin-associated diabetes: Identifying risk factors and physiologic mechanisms
The goal is to understand the mechanism for statin-induced diabetes. We will perform a small clinical trial to assess insulin sensitivity and insulin secretion with gold standard measures before and after statin therapy.

NIH: 1R01DK106236 (PI: Ingelsson) 7/01/16– 6/30/20
Beyond GWAS of insulin resistance: an integrated approach to translate genetic association to function
The goals of this work are to use computational, in vitro and zebrafish models to uncover the function of insulin resistance GWAS variants.
Role: Co-Investigator

Completed Research Support

AHA 5IRG222930034, National Innovative Research Grant (**PI: Knowles**) 1/01/15-12/31/16
Use of electronic phenotyping and machine learning to identify familial hypercholesterolemia in EHRs
The goal of this project is to use cutting edge technologies to find previously undiagnosed FH patients.

AHA 10FTF3360005, National Fellow to Faculty Award (PI: Knowles) 07/01/10-06/30/15
Identification and Characterization of Genetic Determinants of Insulin Resistance
The goal of this project is to replicate finding of our GWAS of insulin sensitivity and to determine the mechanism of action of those variants.

NIH 1 U01 HL107388, Next Gen.Genetic Assoc. Studies (PI: Quertermous) 6/01/12-5/31/17
Identifying the gene networks of insulin resistance: the GENESIPS study
The goal of this project is to recruit and phenotype a large number of subjects with gold standard measures of insulin resistance (IR), ultimately to generate iPSC lines thereby allowing creation of in vitro model systems.
Role: Co-Investigator