Published in final edited form as:

Mol Genet Metab. 2011 ; 104(1-2): 189–191. doi:10.1016/j.ymgme.2011.06.022.

Personalized Genomic Medicine: Lessons from the Exome

Benjamin D. Solomon^{a,*}, Daniel E. Pineda-Alvarez^a, Donald W. Hadley^b, NISC Comparative Sequencing Program^c, Jamie K. Teer^d, Praveen F. Cherukuri^e, Nancy F. Hansen^e, Pedro Cruz^e, Alice C. Young^e, Robert W. Blakesley^e, Brendan Lanpher^f, Stephanie Mayfield Gibson^{g,†}, Murat Sincan^h, Settara C. Chandrasekharappaⁱ, and James C. Mullikin^e

Daniel E. Pineda-Alvarez: pinedaad@mail.nih.gov; Donald W. Hadley: dhadley@nhgri.nih.gov; Jamie K. Teer: teerj@mail.nih.gov; Praveen F. Cherukuri: cherukur@mail.nih.gov; Nancy F. Hansen: nhansen@mail.nih.gov; Pedro Cruz: cruzp@mail.nih.gov; Alice C. Young: alicey@mail.nih.gov; Robert W. Blakesley: rblakesl@nhgri.nih.gov; Brendan Lanpher: BLanpher@cnmc.org; Stephanie Mayfield Gibson: StephanieK.Mayfield@ky.gov; Murat Sincan: murat.sincan@nih.gov; Settara C. Chandrasekharappa: sc84g@nih.gov; James C. Mullikin: mullikin@mail.nih.gov

^aMedical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, 20892, United States of America

^bSocial and Behavioral Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, 20892, United States of America

^cNIH Intramural Sequencing Center, National Human Genome Research Institute, National Institutes of Health, Rockville, Maryland, 20852, United States of America

^dGenetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, 20892, United States of America

^eGenome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, 20892, United States of America

^fDepartment of Genetics and Metabolism, Children's National Medical Center, Washington, DC, 20010, United States of America

^gKentucky Department for Public Health, Frankfort, Kentucky, 40621, United States of America

^hUndiagnosed Diseases Program, National Institutes of Health, Bethesda, Maryland, 20892

ⁱCancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, 20892, United States of America

Abstract

While genomic sequencing methods are powerful tools in the discovery of the genetic underpinnings of human disease, incidentally-revealed novel genomic risk factors may be equally important, both scientifically, and as relates to direct patient care. We performed whole-exome sequencing on a child with VACTERL association who suffered severe post-surgical neonatal pulmonary hypertension, and identified a potential novel genetic risk factor for this complication: a heterozygous mutation in *CPS1*. Newborn screening results from this patient's monozygotic twin provided evidence that this mutation, in combination with an environmental trigger (in this case, surgery), may have resulted in pulmonary artery hypertension due to inadequate nitric oxide

*Corresponding author contact information: Benjamin D. Solomon, MD, NIH, MSC 3717, Building 35, Room 1B-207, Bethesda, MD 20892, Phone: (301)451-7414, Fax: (301)496-7184, solomonb@mail.nih.gov.

†Please note that Stephanie Mayfield Gibson has two last names (Mayfield and Gibson) that should not be hyphenated.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

production. Identification of this genetic risk factor allows for targeted medical preventative measures in this patient as well as relatives with the same mutation, and illustrates the power of incidental medical information unearthed by whole-exome sequencing.

Keywords

Whole-exome sequencing; *CPSI*; pulmonary artery hypertension; VACTERL

1. Introduction

The recent emergence of new sequencing technologies is enabling a revolution in the dissection of the genetic underpinnings of disease. Whole-exome sequencing involves the use of next-generation technologies to sequence all coding regions, or exons, of known genes within the human genome. The exome constitutes slightly more than 1% of the entire genome, but is estimated to contain a large proportion of disease-causing variation, especially in Mendelian disorders. Whole-exome sequencing is efficient, affordable, and has led to remarkable recent scientific achievements [1, 2].

While whole-exome sequencing is a powerful tool in the investigation of genetic disease, this technique also unearths large amounts of genomic risk information, or genetic information that is not related to the primary research target [3]. Managing genomic risk information raises complex issues, but can also be a powerful tool for making important discoveries that were not part of the original research question, as well as for facilitating medical decision-making and health care. We describe such an experience within the context of clinical genetic research in order to demonstrate this point.

Whole-exome sequencing is utilized within our IRB-approved research protocol, which focuses on discovering the causes of VACTERL association, a disorder in which multiple congenital malformations tend to co-occur in a single patient. Typical criteria for diagnosis include at least three of the following: Vertebral anomalies, Anal atresia, Cardiac malformations, Tracheo-Esophageal fistula (TEF), Renal anomalies, and Limb abnormalities [4, 5]. One way we use whole-exome sequencing is to apply it to monozygotic twin pairs in which only one of the twins is affected. In two such twin pairs studied to date, whole-exome sequencing has not revealed any *de novo* discordant exonic variants that are predicted to be pathogenic or obviously related to VACTERL association. However, several variants of interest that may act as susceptibility factors have been identified, and are undergoing further analysis involving expression-based and epigenetic studies (further speculation regarding the pathogenicity of these variants and their connection to VACTERL association remains premature at this point). However, as we demonstrate, the incidental genomic risk information may ultimately be more important to the overall health of participants and their families than should a genetic contribution to VACTERL association be proven: in one patient, review of whole-exome sequencing data revealed the possible cause of a severe neonatal health outcome.

2. Material and Methods

2.1 Subjects

Research participants take part in our IRB-approved study on VACTERL association, with informed consent obtained from all participants; a specific, separate consent is used prior to genomic (including whole-exome) sequencing.

2.2 Whole-exome sequencing and confirmation

We performed whole-exome sequencing of the monozygotic twin pair using DNA extracted from peripheral blood samples (see supplementary material for details). Described variants were confirmed via bidirectional Sanger sequencing (see supplementary material).

3. Results and Discussion

3.1 Clinical Features

One member of a twin pair delivered at 36 4/7 weeks gestation, with a birth weight of 2409 g, was born with a TEF with distal esophageal atresia (EA), a possible ventricular septal defect (very small, membranous, and which appeared to self-resolve), and a dysplastic right kidney. Clinical analysis of this family at the National Institutes of Health Clinical Center (when the proband was approximately 16 months of age) did not suggest an alternate diagnosis other than VACTERL association, and no family members (including his monozygotic twin) were found to have any congenital malformations. As relates to the *CPSI* mutation discussed here, there was no firm family history of pulmonary hypertension or related cardiovascular issues. The proband's father (who was also a *CPSI* mutation carrier) was described as having asthma in childhood. The paternal grandfather (who did not undergo testing) suffered a traumatic childhood injury requiring multiple surgeries, and was reported as "not responding well to anesthetics" in that he would "breathe very heavily", but further details are not known.

The patient underwent routine surgical repair for the TEF/EA on the second day of life (DOL), but his post-operative course was far more complicated than typical [6, 7]. Postoperatively, he suffered severe echocardiogram-documented pulmonary artery hypertension, which was confirmed starting on the day after surgery and on multiple subsequent echocardiograms (the estimated right ventricular pressure was 55–60 mmHg three days after surgery). Because of the hypertension, his medical care required dopamine (DOL 2–14), dobutamine (DOL 2–13), and epinephrine (DOL 2–4) infusions, hydrocortisone (DOL 2–22), sildenafil (DOL 3–21), and milrinone (DOL 7–15), as well as inhaled nitric oxide (NO) (DOL 2–18). He was extubated on DOL 19, and achieved room air on DOL 24; he was discharged from the hospital on DOL 40, with no clinical cardiology follow-up required. No cardiac, pulmonary, or other anomalies that would explain his severe pulmonary artery hypertension were ascertained despite multiple investigations.

3.2 Whole-Exome Sequencing and Confirmation

We are currently investigating multiple genetic factors that may be involved in the underlying malformations in VACTERL association, but none accounts for the pulmonary artery hypertension or would explain why only one twin was affected with VACTERL association. However, whole-exome sequencing identified heterozygosity for a novel mutation in *CPSI* (MIM ID +608307, NM_001875) in both twins, which is likely to account for the proband's susceptibility to severe pulmonary artery hypertension. The variant, c.1589G>T, is predicted to result in a deleterious missense mutation: p.Gly530Val. This variant was not found in 100 ethnically-matched control chromosomes of individuals who underwent whole-exome sequencing through other protocols at the same sequencing center. The amino acid substitution, predicted to be pathogenic by both CDPred (see supplementary material) and PolyPhen-2, occurs in an extremely evolutionarily conserved amino acid residue in the enzyme's bicarbonate phosphorylation domain [8, 9]. This mutation was confirmed via bidirectional Sanger sequencing, and was found to be paternally-inherited (supplementary Fig. 1). Both twins also had a paternally-inherited known single nucleotide polymorphism (SNP) (rs1047891), c.4217C>A, predicted to result in p.Thr1406Asn (supplementary Fig. 2). This known SNP has been extensively investigated in studies of

pulmonary artery hypertension, and there is no evidence that heterozygosity for this SNP would increase risk. Due to differences in nomenclature, note that this SNP is sometimes referred to in the literature as resulting in p.Thr1405Asn) [10–12]. In the affected twin, no additional, discordant genetic variants were identified, including other mutations in the *CPSI* gene or in any other urea cycle or nitric oxide synthetase-related genes.

Homozygous or compound heterozygous mutations in *CPSI* cause Carbamoyl Phosphate Synthetase I Deficiency (CPSID), a rare biochemical disorder involving the urea cycle, the body's essential nitrogen disposal pathway. CPSID typically leads to severe hyperammonemia, often resulting in death or neurocognitive impairment [9, 13]. However, genetic variants in *CPSI* are also associated with pulmonary artery hypertension susceptibility, and may explain our patient's severe post-operative course. The mechanism appears to involve both genetic and environmental factors. On a genetic basis, a loss-of-function *CPSI* mutation is predicted to result in decreased endogenous nitric oxide (NO) availability due to impaired production of the precursors citrulline and arginine, as *CPSI* encodes an enzyme that catalyzes the rate-limiting step of l-citrulline production (Fig.). Lack of NO in turn reduces pulmonary vasculature vasodilatory activity.

In addition to the *CPSI* mutation, an iatrogenic environmental factor is also hypothetically involved here, especially as the patient's twin suffered no similar issues, despite being genotypically identical for the *CPSI* variants. In patients undergoing cardiac surgery, post-operative reduction in citrulline and arginine secondary to cardiopulmonary bypass, has been associated with pulmonary artery hypertension [10–12, 14]. In our patient, another explanation might involve the fact that he was unable to eat (secondary to the TEF) prior to surgery. Most importantly for counseling and medical planning purposes, there is evidence that the risk of pulmonary artery hypertension in carriers of hypomorphic *CPSI* variants can be mitigated by peri-operative administration of the NO metabolites citrulline or arginine [15–17].

3.3 Biochemical Evidence

In order to investigate the hypothetical link between the *CPSI* mutation and the patient's clinical course, we reviewed the proband's and his twin's newborn screening values for citrulline and arginine, as low values of these metabolites would correlate with an increased susceptibility to pulmonary artery hypertension [10–12]. Newborn screening is designed to test for a variety of potentially treatable genetic and metabolic disorders, including certain biochemical disorders that manifest with elevated levels of citrulline or arginine due to abnormally functioning urea cycle enzymes downstream of the *CPSI* enzyme. The proband underwent newborn screening post-surgically, shortly after receiving transfusions, and while also receiving total parenteral nutrition (TPN). While these tests did not show evidence of an inborn error of metabolism, the results were difficult to interpret because of necessary medical interventions, and were in fact labeled as “invalid” due to transfusions. However, we were able to examine the monozygotic twin's newborn screening results, as he carried the same *CPSI* mutation and did not require medical interventions. (It must be noted that this evidence is admittedly extremely circumstantial as relates to the link between the proband's pulmonary hypertension and the *CPSI* mutation.) The unaffected twin's citrulline and arginine levels were 9.0 $\mu\text{mol/L}$ (mean 13.4 $\mu\text{mol/L}$), and 7.0 $\mu\text{mol/L}$ (mean 12.1 $\mu\text{mol/L}$), respectively. Compared to approximately 42,000 infants who underwent newborn screening up to 48 hours after birth in the same year in the same state laboratory, both values are between 1 and 2 standard deviations below the mean, which concords with previous research measuring these analytes in relation to risk for pulmonary artery hypertension [10]. As a comparison, his phenylalanine level on the same newborn screening test was 52.0 $\mu\text{mol/L}$, which is approximately the mean for 42,000 tested infants.

In summary, whole-exome sequencing revealed a novel mutation in the gene *CPSI*. There is no evidence that this mutation is related to the pathogenesis of VACTERL association, although it is interesting to consider how impaired nitric oxide production in early fetal development might be related to certain types of congenital malformations. While additional, unidentified genetic and/or environmental factors may be etiologically involved, the *CPSI* mutation might have contributed to a patient's severe clinical course in regards to pulmonary hypertension. It is additionally exceedingly unlikely that the presence of this mutation would have been considered by even the most astute clinician. However, knowing that this patient, as well as his paternal relatives, may be at risk for pulmonary artery hypertension during surgery, could allow for a specific, targeted biochemical preventative treatment to reduce morbidity in the future. This illustrates the use of personalized genomic medicine in the context of genomic risk information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

CDPred	refers to pathogenicity-predicting software used for exome analysis
CPSID	Carbamoyl Phosphate Synthetase I Deficiency
DOL	day-of-life
EA	esophageal atresia
IRB	Institutional Review Board
NO	nitric oxide
SNP	single-nucleotide polymorphism
TEF	trachea-esophageal fistula
VACTERL	vertebral anomalies, anal atresia, cardiac malformations, tracheo-esophageal fistula; renal anomalies; limb abnormalities

Acknowledgments

This research was supported by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health. We would like to extend our gratitude to the family for participating in this study. Sanger sequencing support was provided by the core DNA sequencing facility, National Institute of Neurological Disorders and Stroke. We would like to thank Dr. William A. Gahl for use of data (collected through the National Institutes of Health Undiagnosed Diseases Program) as controls. We would also like to thank Dr. Maximilian Muenke for his mentoring and support.

References

1. Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ. Exome sequencing identifies the cause of a mendelian disorder. *Nat. Genet.* 2010; 42:30–35. [PubMed: 19915526]
2. Teer JK, Mullikin JC. Exome sequencing: the sweet spot before whole genomes. *Hum. Mol. Genet.* 2010; 19:R145–R151. [PubMed: 20705737]
3. Tucker T, Marra M, Friedman JM. Massively parallel sequencing: the next big thing in genetic medicine. *Am. J. Hum. Genet.* 2009; 85:142–154. [PubMed: 19679224]
4. Quan L, Smith DW. The VATER association. Vertebral defects, Anal atresia, T-E fistula with esophageal atresia, Radial and Renal dysplasia: a spectrum of associated defects. *J. Pediatr.* 1973; 82:104–107. [PubMed: 4681850]

5. Solomon BD, Pineda-Alvarez DE, Raam MS, Bous SM, Keaton AA, Velez JI, Cummings DA. Analysis of component findings in 79 patients diagnosed with VACTERL association. *Am. J. Med. Genet. A.* 2010; 152A:2236–2244. [PubMed: 20683998]
6. Holland AJ, Fitzgerald DA. Oesophageal atresia and tracheo-oesophageal fistula: current management strategies and complications. *Paediatr. Respir. Rev.* 2010; 11:100–106. quiz 106–107. [PubMed: 20416546]
7. Spitz L. Oesophageal atresia. *Orphanet J. Rare Dis.* 2007; 2:24. [PubMed: 17498283]
8. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. *Nat. Methods.* 2010; 7:248–249. [PubMed: 20354512]
9. Haberer J, Shchelochkov OA, Wang J, Katsonis P, Hall L, Reiss S, Eeds A, Willis A, Yadav M, Summar S, Lichtarge O, Rubio V, Wong LJ, Summar M. Molecular Defects in Human Carbamoyl Phosphate Synthetase I: Mutational Spectrum, Diagnostic and Protein Structure Considerations. *Hum. Mutat.* 2010
10. Pearson DL, Dawling S, Walsh WF, Haines JL, Christman BW, Bazyk A, Scott N, Summar ML. Neonatal pulmonary hypertension--urea-cycle intermediates, nitric oxide production, and carbamoyl-phosphate synthetase function. *N. Engl. J. Med.* 2001; 344:1832–1838. [PubMed: 11407344]
11. Summar ML, Gainer JV, Pretorius M, Malave H, Harris S, Hall LD, Weisberg A, Vaughan DE, Christman BW, Brown NJ. Relationship between carbamoyl-phosphate synthetase genotype and systemic vascular function. *Hypertension.* 2004; 43:186–191. [PubMed: 14718356]
12. Canter JA, Summar ML, Smith HB, Rice GD, Hall LD, Ritchie MD, Motsinger AA, Christian KG, Drinkwater DC Jr, Scholl FG, Dyer KL, Kavanaugh-McHugh AL, Barr FE. Genetic variation in the mitochondrial enzyme carbamyl-phosphate synthetase I predisposes children to increased pulmonary artery pressure following surgical repair of congenital heart defects: a validated genetic association study. *Mitochondrion.* 2007; 7:204–210. [PubMed: 17188582]
13. Hoshida R, Matsuura T, Haraguchi Y, Endo F, Yoshinaga M, Matsuda I. Carbamyl phosphate synthetase I deficiency. One base substitution in an exon of the CPS I gene causes a 9-basepair deletion due to aberrant splicing. *J. Clin. Invest.* 1993; 91:1884–1887. [PubMed: 8486760]
14. Barr FE, Beverley H, VanHook K, Cermak E, Christian K, Drinkwater D, Dyer K, Raggio NT, Moore JH, Christman B, Summar M. Effect of cardiopulmonary bypass on urea cycle intermediates and nitric oxide levels after congenital heart surgery. *J. Pediatr.* 2003; 142:26–30. [PubMed: 12520250]
15. Smith HA, Canter JA, Christian KG, Drinkwater DC, Scholl FG, Christman BW, Rice GD, Barr FE, Summar ML. Nitric oxide precursors and congenital heart surgery: a randomized controlled trial of oral citrulline. *J Thorac Cardiovasc Surg.* 2006; 132:58–65. [PubMed: 16798303]
16. Badesch DB, Abman SH, Ahearn GS, Barst RJ, McCrory DC, Simonneau G, McLaughlin VV. Medical therapy for pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. *Chest.* 2004; 126:35S–62S. [PubMed: 15249494]
17. Schulze-Neick I, Penny DJ, Rigby ML, Morgan C, Kelleher A, Collins P, Li J, Bush A, Shinebourne EA, Redington AN. L-arginine and substance P reverse the pulmonary endothelial dysfunction caused by congenital heart surgery. *Circulation.* 1999; 100:749–755. [PubMed: 10449698]

Fig.

Hypothesized explanation for pulmonary artery hypertension in the patient described here [10–12, 14]. A loss-of-function mutation in the *CPS1* gene (1) leads to impaired production of citrulline (2) and arginine (3). The lack of availability of these precursors leads to decreased availability of nitric oxide (4), resulting in decreased pulmonary artery vasodilation, which becomes especially severe in concert with iatrogenic depletion of arginine and citrulline.