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Molecular Diagnosis of Long-QT syndrome at 10 Days of Life by Rapid Whole Genome Sequencing

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Abstract

Background—The advent of clinical next generation sequencing is rapidly changing the landscape of rare disease medicine. Molecular diagnosis of long QT syndrome (LQTS) can impact clinical management, including risk stratification and selection of pharmacotherapy based on the type of ion channel affected, but results from current gene panel testing requires 4 to 16 weeks before return to clinicians.

Objective—A term female infant presented with 2:1 atrioventricular block and ventricular arrhythmias consistent with perinatal LQTS, requiring aggressive treatment including epicardial pacemaker, and cardioverter-defibrillator implantation and sympathectomy on day of life two. We sought to provide a rapid molecular diagnosis for optimization of treatment strategies.

Methods—We performed CLIA-certified rapid whole genome sequencing (WGS) with a speed-optimized bioinformatics platform to achieve molecular diagnosis at 10 days of life.

Results—We detected a known pathogenic variant in *KCNH2* that was demonstrated to be paternally inherited by followup genotyping. The unbiased assessment of the entire catalog of human genes provided by whole genome sequencing revealed a maternally inherited variant of unknown significance in a novel gene.

Conclusions—Rapid clinical WGS provides faster and more comprehensive diagnostic information by 10 days of life than standard gene-panel testing. In selected clinical scenarios such as perinatal LQTS, rapid WGS may be able to provide more timely and clinically actionable information than a standard commercial test.

Keywords

pediatrics; genetics; genomics; Long QT syndrome; perinatal; whole genome sequencing

Introduction

The Long QT syndrome (LQTS) results from abnormalities in trans-membrane ion channels and accessory proteins which prolong the repolarization phase of the cardiac action potential, and predispose patients to fatal cardiac arrhythmias. Prolongation of the QT interval may rarely present in the perinatal period as bradycardia with 2:1 functional AV block secondary to prolonged ventricular refractory period with torsade de pointes, and may account for a non-trivial percentage of intrauterine fetal demise (1-3). In a small longitudinal study of perinatal LQTS five of ten subjects with fetal arrhythmias experienced sudden death or cardiac arrest(3). The physical removal of beta-adrenergic stimulus to the heart by left-sympathetic ganglionectomy has been reserved for patients with the most severe arrhythmias, and is often accompanied by pacemaker and ICD placement(3,4). The association of perinatal LQTS with intrauterine fetal demise and overall poor survival suggests a need for therapeutic interventions, which might be guided by early molecular diagnosis(2,3,5).

Case Report

A 38 week and 4 day gestational age female infant weighing 3.38 kg was delivered by caesarean section for fetal bradycardia noted at a routine obstetrics visit. Following delivery APGAR scores were 8 and 9 with an irregular heart rate and bradycardia. The initial ECG revealed a PR prolongation of 165 ms, QRS prolongation of 105 ms, and a corrected QT interval prolongation of 607 ms (Bazett's formula), with intermittent 2:1 functional atrioventricular (AV) block secondary to the significantly prolonged ventricular refractory period (Figure 1A). The baby then developed recurrent runs of polymorphic wide-complex tachycardia with hemodynamic compromise prompting chest compressions (Figure 1C). A structurally normal heart was demonstrated on echocardiogram. The initial dose of intravenous lidocaine resulted in a small QT shortening (677 to 639 ms), and lidocaine and esmolol drips were administered initially resulting in more consistent 1:1 AV conduction. Blood was drawn for rapid whole genome sequencing on day of life (DOL) 1, due to the high suspicion for long QT syndrome (LQTS).

Over the next 24 hours, the baby continued to have episodes of 2:1 block and multiform ventricular ectopy with hemodynamic compromise. Given the refractory nature of the 2:1 block and continued ventricular ectopy to multiple doses of lidocaine (1 mg/kg), esmolol (500 mcg/kg), and magnesium (25 mg/kg) a multidisciplinary team determined the infant was at imminent risk and chose to place an implantable cardioverter/defibrillator (ICD) to restore AV synchrony and performed a left sympathetic ganglionectomy to reduce adrenergic stimulus. Via a median sternotomy, the left pleural space was entered and the left sympathetic chain from the lower half of the stellate ganglion to T5 was removed. A bipolar ventricular lead was placed on the ventricular apex and a bipolar atrial lead placed on the lateral wall of the right atrium. The defibrillator coil was positioned in the posterior pericardial space, and fixed to the pericardium at the left atrial appendage. The device pocket was created in the right upper abdomen (Figure 1B). Adequate sensing and pacing thresholds were confirmed, but defibrillation threshold testing could not be performed. The

patient tolerated the procedure well without complications or clinical suggestion of Horner's syndrome.

Following surgery the patient was paced in DDD mode at a base rate of 130 bpm with 1:1 AV-synchronous pacing, and remained free from further arrhythmias. On DOL 3 the patient was extubated and transitioned to mexiletine 3 mg/kg per day and propranolol 1mg/kg per day while anti-arrhythmic drips were titrated downward to cover the possible molecular etiologies of potassium channel or sodium channel related LQTS. There was clinical concern for seizures on DOL 7, however an EEG did not reveal epileptiform activity and there was no further clinical indication of neurological deficit. Automated auditory brainstem response testing was normal and she was discharged from the hospital to the care of her parents on DOL 11.

Family history revealed that the infant was the product of a normal pregnancy to a 20 year old mother and 21 year old father of Hispanic descent. Consanguinity was denied. The maternal family history was benign, with no reports of syncope, seizures, SIDS or stillbirth, sudden cardiac death, heart failure, congenital heart disease, accidental or unexplained death, death before age 50, ICD or pacemaker implantation, deafness, episodic paralysis, or syndactyly. In contrast the paternal family history was notable for a paternal great uncle who died suddenly in childhood (Figure 1C). The parental ECGs revealed a supine QTc of 440 ms and standing QTc of 457 ms in the father, and a supine QTc 383 of standing QTc of 406 ms in the mother.

Methods

Ethics Statement

The Stanford Administrative Panels for the Protection of Human Subjects approved all research described herein which was conducted under the guidelines of the declaration of Helsinki. The patient's parents provided written informed consent on behalf of themselves and their infant daughter for participation in this study, in addition to receiving counseling from physicians and genetic counselors about the risks and benefits of genome sequencing. In pre-test counseling, the parents consistently expressed a verbal desire not to be informed of incidentally discovered genetic information regarding the long-term risks of other diseases such as cancer, neurodegenerative disorders, or cardiovascular disease outside of the arrhythmia under treatment. The expressed verbal desire was confirmed individually by both parents in the written consent process as a checkbox on the consent form. The parents were informed that this additional genetic information would remain available to them and the patient, should their desire for information change at a later date.

Genetic Analysis

For complete details of whole genome sequencing, bioinformatics analysis, and followup parental genotyping, please refer to the supplementary methods. In brief, data was aligned and single nucleotide (SNVs) and insertion or deletion variants (indels) called using rtgVariant version 1.0.2 (Real Time Genomics Inc., San Bruno, California). Variants were annotated with a custom database and pipeline providing annotation from 97 data sources

including allele frequency estimation, classification of predicted variant function by location, evolutionary conservation, gene function and pathway, phenotypic association, and standard predictions of altered protein function (supplementary methods). To focus on *de novo* or rare protein altering variants consistent with a rare disease, we excluded variants occurring within segmental duplications and variants with a minor allele frequency (MAF) of more than 0.02 in the 1000 Genomes Project April, 2012 release or the Complete Genomics 69 public genomes databases. We searched HGMD, OMIM, ClinVar, and the NHGRI genome wide association study (GWAS) catalog for terms relating to inherited arrhythmias, cardiomyopathies, and the cardiac action potential to define a list of 451 genes with a high pre-test probability for involvement in LQTS (6-8). Rare protein altering variants across the entire genome were examined manually with particular attention to the gene list with evidence for involvement in inherited arrhythmias or the cardiac action potential. Confirmation of variants of interest was carried out with PCR amplification and direct sequencing or restriction enzyme analysis.

Results

Whole Genome Sequencing, Genetic Analysis, and Parental Genotyping

The unprocessed whole genome sequence data passing QC totaled 1,239,367,160 reads of 100 base pair length. Read mapping and variant calling with rtgVariant v1.0.2 to the University of California, Santa Cruz human genome reference sequence (hg19) aligned 88% of the reads yielding a 32.26 mean read depth. Filtering called sites with a read depth of 8 and automated variant recalibration score of 0.02 yielded 3,711,590 single nucleotide variants with a transition to transversion ratio of 2.08 and 754,196 insertions and deletions (supplementary table 1). Within the list of 451 genes noted above we observed the median coverage depth was 39 while the number of bases covered at less than 8× was 2,133 bases or 0.06% suggesting excellent sensitivity for detection of genetic variants. The analysis was completed in 8.6 days (supplementary table 2).

Coding Mutations—Annotating the variants and applying our filtration criteria for rare disease yielded 909 protein altering mutations. Manual inspection of the call set revealed the patient to be heterozygous for three rare exonic variants related to inherited arrhythmias or the cardiac action potential. A rare heterozygous nonsynonymous variant previously associated with neonatal presentation of LQTS2 was observed in *KCNH2* NM_172056:c.1838C>T (p.T613M, rs199473524) and a novel heterozygous substitution/frameshift variant was observed in *RNF207* NM_207396:c.1807-1811>GCC (p.G603fs*). The *KCNH2* variant has been associated with LQTS in at least 5 unrelated individuals and demonstrated strong co-segregation evidence in all four families reported (supplementary table 3). In addition the variant is absent from approximately 6,555 published controls and individuals from publicly-available population datasets. The mutation occurs in the pore region and functional studies support a dominant negative effect preventing normal trafficking of the HERG protein to the surface membrane and decreasing I_{Kr} , the rapidly activating delayed rectifier potassium current. Sanger sequencing revealed that this *KCNH2* variant was paternally inherited while restriction enzyme analysis showed the *RNF207* variant was maternally inherited (Figure 2).

A novel heterozygous nonsynonymous variant was observed in *CACNA1D* NM_000720: c.G362>A (p.S121N), a subunit of the L type calcium channel and was paternally inherited (supplementary figure 1). It is difficult to ascribe the perinatal LQTS to this variant as the human reports of *CACNA1D* has been associated with a prolonged PR interval in two pedigrees, while the knockout mouse does not display QT prolongation (9). The observed variant occurs in the cytoplasmic topological domain significantly closer to the N-terminus than the two reported mutations which occur in transmembrane helical domains, and was inherited from the father who had a normal PR interval of 120 ms. The patient also displayed a novel frameshift variant in *MYH6* NM_002471:c.3849delG (p.Q1283*fs) which was maternally inherited (supplementary figure 1). *MYH6* has been linked to atrial septal defects and other forms of structural congenital heart disease, dilated cardiomyopathy, and also sick sinus syndrome(10-13). Echocardiography demonstrated a structurally normal heart with normal functional parameters. Prolongation of the PR interval is not a recognized component of neonatal LQTS, and we cannot exclude that the *CACNA1D* or *MYH6* variants may also contribute to the prolonged PR or QT intervals in this patient.

To remain consistent with the expressed parental desires and the joint AAP/ACMG guidelines covering predictive genetic testing in children for adult-onset diseases, we report here only the generalities of our incidental findings within the list of 56 genes recently suggested by the ACMG (14,15) Applying the rare disease filtering criteria (minor allele frequency of 0.02 or greater in the 1000 Genomes Project April, 2012 release or the Complete Genomics 69 public genomes databases) to the list of 56 genes yields only one uncommon protein altering variant other than *KCNH2*. Not occurring in an arrhythmia related gene, the variant is uncommon in aggregate but displays a minor allele frequency of 0.04 within the 1000 genomes admixed American population, from which the patient traces her ancestry. Based on minor allele frequency the variant is unlikely to be of clinical significance.

Discussion

In contrast with the speed of current gene panel testing, we present here the earliest CLIA diagnosis of LQTS at 10 days of age in one of the smallest patients to receive an epicardial ICD and left stellate ganglionectomy(16). The speed of sequencing and analysis allowed a CLIA molecular diagnosis to be reached before hospital discharge on DOL 11, which enabled gene-specific drug therapy for this infant's condition weeks earlier than would have been possible with standard clinical genetic testing. Our early and invasive intervention was prompted by the persistence of medically refractory arrhythmias, but by the standards of adult electrophysiology there is little true evidence to guide therapy in this patient population as perinatal LQTS is an infrequent occurrence even at large referral centers. Evidence based intervention is further limited by the intervention bias is inherent in all retrospective procedural studies such as that of Aziz et al. (3,5).

Clinical heterogeneity is common in LQTS as multiple family members carrying the same mutation may have different QT intervals and clinical manifestations(17). Such heterogeneity is presumed to arise from variation at other loci throughout the genome(18) as well as environmental triggers. The *KCNH2* T613M variant found in our patient

(rs199473524) has been previously associated with a clinical presentation of LQT2 as 2:1 heart block *in utero* or in infancy (supplementary table 3), but in our report was inherited from a clinically asymptomatic father with a supine QTc of 440ms and standing QTc of 457 ms both in the normal range.

We hypothesize that the *RNF207* variant combines in a bi-genic fashion with a primary *KCNH2* T613M variant which impacts channel trafficking to cause disease in the patient. Alternative candidates for a second variant might include additional maternally inherited regulatory variants in *KCNH2*, other *de novo* variants, or maternally inherited variants in genes related to ubiquitination or protein trafficking. There are no regulatory variants proximal to *KCNH2* which display convincing evidence of transcriptional regulation of this gene (supplementary results, supplementary table 4). *De novo* mutations are likely to be novel, and further stringent sorting of all protein altering mutations reveals only 187 novel mutations within this patient, of which only *RNF207*, *CACANA1D*, and *MYH6* are known to be involved in the cardiac action potential and the inheritance pattern investigated in our trio. We cannot exclude the possibility that the other genes, some of which are likely *de novo* mutations, have an unrecognized role in modulating the cardiac action potential. Finally looking more carefully at the function of these 187 genes, four of the genes have a role in the ubiquitination pathway *MAP3K1*, *FBXW7*, *HERC1*, and *RNF207*. The predicted function of *RNF207* is a RING-type zinc finger, a class of protein which interacts with the ubiquitination pathway as E3 ubiquitin-protein ligases. Thus, it is plausible that disordered ubiquitination displayed by the *RNF207* Q1283*fs further compounds the known channel trafficking defect of the *KCNH2* T613M mutation. Functional investigation to investigate any interaction between *KCNH2* and *RNF207* is necessary.

Whole genome sequencing provides an unbiased assessment of the entire complement of human genes, including loci not covered on commercial gene panel testing for inherited arrhythmias. The variant in *RNF207* found in our patient, for example, would not have been assessed or reported in commercial panel testing, which is limited to approximately one dozen genes pathogenic for LQTS. Evidence for an association between *RNF207* and the QT interval is robust. Common variants within the transcribed coding regions of *RNF207* are independently linked to prolongation of the QT interval with high statistical confidence by two large GWAS studies and the particular *RNF207* splice isoform containing the final exon where the observed mutation is located is highly transcribed in adult heart (19-21). The novel variant p.G603fs* directly overlaps rs846111 (p.G603A) identified independently by both GWAS studies. Patients carrying a higher burden of mutations in canonical LQT genes appear to have a longer QTc at presentation and an increased risk of serious cardiac events(22). Thus our patient may have inherited a second variant impacting the QT interval from her mother, and a bi-genic inheritance may contribute to explaining the early and severe perinatal presentation of LQTS in the infant. By moving beyond the confines of today's clinical testing panels, whole genome sequencing will yield more genetic data on each patient which will be a useful substrate for the discovery of modifier genes and may eventually lead to better risk-stratification for affected family members.

More broadly, this case illustrates the direct clinical application and immediate clinical impact of rapid whole genome sequencing on the diagnosis and treatment of a well

characterized genetic disease. This stands in contrast to earlier reports of applied clinical sequencing within pediatrics, which have focused most successfully on diagnostic mysteries (23). Importantly, treatment decisions, lifestyle-modification, and risk-stratification based on the molecular subtype of LQTS are now commonly made (24), yet actionable results from CLIA-certified commercial gene-panel testing for arrhythmia genes have turn-around times from 4 to 16 weeks depending on the provider (supplementary table 5). Even then, currently-available panels are only able to identify a causative variant in 72% of tested patients (25). For our patient, whole genome sequencing successfully achieved a CLIA-certified laboratory diagnosis within days prior to hospital discharge.

Molecular understanding of the patient's disease allows for rational drug therapy optimized to the root cause of the prolonged ventricular refractory period, whether a defective sodium channel or potassium channel(26,27). Initially, the slight shortening of our patient's QTc observed with lidocaine raised suspicion for LQT3 and encouraged a clinical focus on sodium channel blockade with lidocaine transitioned to mexiletine. Distinguishing the underlying molecular pathophysiology as the result of a defect in potassium channel trafficking and IK_r rather than sodium entry allows for discontinuation of the sodium channel inhibitor mexiletine. Like many anti-arrhythmics mexiletine has narrow therapeutic index and overdose is known to cause seizures(28-31). Inadvertent dosing errors of medications compounded for infants in suspension form is not an uncommon occurrence, however it is difficult to estimate the incidence of serious toxicities of drugs that are infrequently applied to the neonatal population (32,33). In place of sodium channel blockade, evidence from the adult literature would favor agents such as beta blockers known to be effective in preventing sudden death in LQT2(34). After excluding a sodium channel mutation with rapid whole genome sequencing, the mexiletine was discontinued and the treatment paradigm appropriately oriented to beta-adrenergic receptor blockade weeks earlier than would have been possible with standard genetic testing.

Family members may also benefit from the timely results of a genetic test, which may prompt clinical screening and cascade genetic testing. Though the parental genotypes still require confirmation in a CLIA-certified laboratory, a pathogenic *KCNH2* genotype in the father suggests that he will require long-term cardiology follow-up and treatment with beta-blockers. Even in cases like this one where analysis was limited to a specific genetic question, comprehensive genomic tests such as whole genome sequencing will also uncover uncertain findings. For instance, the discovery of the *RNF207* variant in the mother is more complex. Rare deleterious mutations in *RNF207* have not been routinely assayed in patients with clinical evidence of LQTS and therefore have not been previously reported to cause or to exacerbate life-threatening disease; however, the literature suggests that common coding variation in this gene has a small but measurable impact on the QTc. It is therefore reasonable to hypothesize that a rare protein truncating variant in *RNF207* may have a more significant impact on the QTc, conservatively we would classify this as a highly suspicious variant of unknown significance (VUS). Additionally the function of *RNF207* within the ubiquitin pathway may yield a particularly deleterious interaction with the T613M mutation which causes trafficking defects of the *KCNH2* protein product. The clinical implications of this genetic finding in isolation found in the patient's mother is not known, however our

current institutional practice (as outlined in supplementary table 6) for unaffected relatives of patients with LQTS carrying a VUS is to perform periodic screening ECGs. Information on genetic findings of uncertain significance derived from whole genome sequencing is likely to change as knowledge of the impact of novel loci or phenotypic modifiers is improved. As in all genetic testing, the discovery of novel loci and investigation of their inheritance patterns of the disease of interest may still prompt additional testing and medical care not anticipated by the care providers or the family members(35).

Conclusion

In an infant with malignant ventricular arrhythmia secondary to LQTS we performed CLIA-certified whole genome sequencing and found a known pathogenic variant in *KCNH2* at 10 days of life, clarifying the pathophysiological basis of disease in this infant and allowing pharmacotherapy to be tailored to the molecular diagnosis prior to hospital discharge. We hypothesize that a maternally inherited novel VUS in *RNF207* may have contributed to the early and severe presentation. This infant was among the youngest children to receive an implantable defibrillator and therapeutic sympathectomy at 2 days of life. In cases of malignant perinatal presentation of LQTS, rapid whole genome sequencing may provide more information at an earlier time than is currently possible with standard gene-panel testing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

WGS	Whole Genome Sequencing
LQTS	Long QT syndrome
QTc	Corrected QT interval
ICD	Implantable Cardioverter/Defibrillator
DOL	Day of life
EEG	Electroencephalogram
ECG	Electrocardiogram
CLIA	Clinical Laboratory Improvement Amendments
DNA	Deoxyribonucleic acid
HGMD	Human Gene Mutation Database
OMIM	Online Mendelian Inheritance in Man
NHGRI	National Human Genome Research Institute
QC	Quality control
AAP	American Academy of Pediatrics
ACMG	American College of Medical Genetics

GWAS Genome Wide Association Study

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Clinical Perspectives

Clinical genetic testing for inherited arrhythmias and other genetic disorders is currently limited to a panel of selected genes for a related group of conditions. Panel testing may yield a plausible variant in approximately 2/3 of tested patients with LQTS, but does not always explain the variability in presentation within family members. Comprehensive genetic testing, in the form of whole exome sequencing (WES) and whole genome sequencing (WGS) is now available in the clinic which begs the question when considering panel testing for an arrhythmia, what is the clinical scenario that takes advantage of a faster and more comprehensive genetic test? Our report illustrates that in a case of perinatal LQTS, rapid WGS yielded an actionable molecular diagnosis weeks earlier than traditional panel testing, and also provided clues about the severe presentation of the patient. The sensitivity and specificity of WES and WGS compared to panel genetic testing has yet to be fully established, but in cases without an obvious diagnosis the results of comprehensive testing may be stored and revisited in the future as novel causative genes are identified. The cost of clinical WES and WGS continues to decline approaching the cost of panel genetic testing. We suggest that additional demonstrations of comprehensive genetic testing are needed to highlight the clinical scenarios in inherited cardiovascular disease such as LQTS where WES and WGS may provide a more accurate, timely, or comprehensive diagnosis than panel genetic testing.

Figure 1. Clinical Characteristics of an Infant Presenting with Long-QT Syndrome

Panel A shows an ECG from the time of presentation demonstrating PR prolongation of 165 ms, QRS prolongation of 105 ms, QTc of 607 ms, and intermittent 2:1 functional atrioventricular (AV) block due to a prolonged ventricular refractory period. Panel B is a representative chest radiograph on DOL 5 demonstrating the surgical positioning of the pacing leads, defibrillator coil, and generator. Panel C displays a three-generation pedigree notable for the unexplained childhood death of a paternal great uncle. Panel D is a rhythm strip with pulse oximetry tracing demonstrating polymorphic ventricular arrhythmia.

Figure 2. Familial Inheritance of Protein Altering Variants Implicated in Abnormal Ventricular Repolarization

A known disease causing missense mutation in *KCNH2* is paternally inherited, and a heterozygous signal in the patient and father is indicated by a red arrow on the chromatogram. A novel frameshift mutation in *RNF207*, a gene independently identified in two GWAS studies to impact the QT interval, is maternally inherited as evidenced by the abrogation of an HaeIII restriction site in a PCR product and presence of a protected band in the patient and mother but not the father.