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Array Comparative Genomic Hybridization Identifies a Heterozygous Deletion of the Entire *KCNJ2* Gene as a Cause of Sudden Cardiac Death

Running title: *Marquis-Nicholson et al.; KCNJ2 whole gene deletion*

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Abstract:

Background - Large gene re-arrangements, not detectable by standard molecular genetic sequencing techniques, are present in a minority of patients with long QT syndrome (LQTS). We aimed to screen for large rearrangements in genes responsible for LQTS as part of the molecular autopsy of a 36 year old woman who died suddenly and had a negative autopsy. A retrospective analysis of an ECG identified a long QT interval, but sequencing of known LQT genes was uninformative.

Methods and Results - Array comparative genomic hybridization (aCGH) was used to screen for deletions and duplications in 101 genes implicated in cardiac disorders and sudden death using a post-mortem blood sample. A 542kb deletion encompassing the entire *KCNJ2* gene was identified in the decedent. The mother had electrocardiographic U wave changes consistent with Andersen-Tawil Syndrome (ATS) and exaggerated by exercise, but none of the characteristic non-cardiac features. Fluorescence *in situ* hybridization (FISH) confirmed the deletion in the decedent and established its presence in the mother.

Conclusions - A novel application of aCGH and FISH has identified that LQTS and sudden cardiac death may occur as a result of a deletion of an entire gene. The case also supports recent research suggesting that non cardiac features of ATS occur only with missense or minor gene rearrangements in *KCNJ2* resulting in a dominant negative effect on Kir2.x channels.

Keywords: sudden cardiac death, long QT syndrome, cardiac arrhythmia, Andersen-Tawil syndrome, comparative genomic hybridization, molecular diangostic techniques

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Introduction

Thirty to 50% of sudden cardiac deaths in the young (1-40 years of age) are due to an inherited cardiac abnormality even when a forensic autopsy reveals no overt cause of death.[1-4] These diagnoses can be reached either through cardiac/genetic investigation of relatives of the deceased and/or through a molecular autopsy. Between 16-28% of such cases are due to long QT syndrome.[1-4]

Large gene rearrangements, which are not detected by standard molecular genetic sequencing, have recently been discovered to underlie a minority of cases of severe forms of long QT syndrome types 1 and 2.[5-7] No previous studies have screened for such changes in autopsy-negative sudden death. The screening of LQTS genes for deletion and duplication mutations has largely used a technique termed Multiplex Ligation-Dependent PCR Amplification (MLPA); however, for enhanced gene coverage we transitioned to a technique known as array Comparative Genomic Hybridization (aCGH), which has not been previously applied to inherited heart diseases.[8-10]

We describe for the first time a unique heterozygous whole-gene deletion of the *KCNJ2* gene, detected using aCGH at molecular autopsy of a 36 year old woman who suffered a sudden unexpected nocturnal death. She had a previous history of "drop attacks" with seizures with negative neurological investigations. She was found retrospectively to have marginal QT prolongation on an incidental ECG. One copy of the *KCNH2* gene was proven to be absent in the mother using Fluorescence *in situ* hybridization (FISH). The mother has a long QT-U complex typical of Andersen Tawil syndrome Andersen-Tawil Syndrome (ATS). Despite the heterozygous absence of the entire gene, neither the decedent nor the mother had any other phenotypic features of ATS.

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Methods

Deletion/duplication analysis – array comparative genomic hybridization (aCGH)

Genomic DNA (gDNA) was extracted from peripheral blood EDTA samples using the Gentra Puregene DNA Extraction kit (Qiagen), according to manufacturer's instructions. The analysis of the proband, and parents, was undertaken as part of a coronial investigation and clinical follow-up, respectively, and proceeded according to informed consent and Health and Disability Ethics Committee approval (reference AKX 02/00/107).

A Roche NimbleGen 12x135K Custom CGH Array was used for deletion/duplication analysis. This bespoke CGH array was designed to interrogate the coding regions of 101 genes associated with a range of cardiac and neuromuscular disorders, including those responsible for LQT1(*KCNQ1*), LQT2 (*KCNH2*), LQT3 and Brugada syndrome (*SCN5A*, *SCN1B*, *SCN3B*, *GPD1L*), LQT4 (*ANK2*), LQT5 (*KCNE1*), LQT6 (*KCNE2*), ATS/LQT7 (*KCNJ2*), Timothy syndrome/LQT8 (*CACNA1c*), LQT9 (*CAV3*), LQT10 (*SCN4B*), LQT11 (*AKAP9*) and LQT12 (*SNTA1*). See Supplemental Material for the full gene list. The probe densities in exons and introns, as well as extra-genic regions of the human genome, have been described elsewhere.[8-10]

Two hundred and fifty nanograms of gDNA were processed according to the manufacturer's instructions (NimbleGen Array User's Guide: CGH and CNV Arrays v6.0; http://www.nimblegen.com). In brief, extracted gDNA from samples and Promega controls was denatured in the presence of a Cy3- (test) or Cy5- (control) labelled random primers and incubated with the Klenow fragment of DNA polymerase, together with dNTPs (5mM of each dNTP), at 37ºC for 2 hours. The reaction was terminated by the addition of 0.5M EDTA (21.5µL), prior to isopropanol precipitation and ethanol washing. Following quantification, the

test and sex-matched control samples were combined in equimolar amounts and applied to one of twelve arrays on the microarray slide. Hybridization was carried out in a Roche NimbleGen Hybridization Chamber for a period of 48 hours. Slides were washed and scanned using a NimbleGen MS 200 Microarray Scanner. Array image files (.tif) produced by the MS 200 Data Collection Software were imported into DEVA v1.2.1 (Roche NimbleGen Inc) for analysis. Each genomic region exhibiting a copy number change within the genes of interest was examined using the UCSC genome browser (http://genome.ucsc.edu/) to determine the location and significance of the change. Data was filtered using a $\log_2$ratio threshold of less than -0.4 over 6 probes for a deletion and greater than 0.4 over 15 probes for a duplication.

Fluorescence *in situ* hybridization (FISH)

Peripheral blood samples from the proband's parents were collected in heparin and cultured according to standard cytogenetic protocols. BAC probes RP11-633A13 (hg19 coordinates: chr17:67869421-68051742) and RP11-693H11 (hg19 coordinates: chr17:68035454-68237499) were chosen from the Human BAC DNA library-32K set. These probes were labelled with orange fluorescent dye. Clone CEP17 was labelled with green (Vysis), and was used as an internal control. The FISH method followed the procedure of Pinkel *et al.*[11] with some modifications. Co-denaturation was achieved by placing the slides into thermal cycler (PTC-200) preheated to 87ºC for 2 minutes. The slides were hybridized overnight in a humidified chamber at 37 ºC. The following day, the slides were subjected to a stringency wash in 0.4xSSC at 74 ºC for 2 minutes followed by 2xSSC at room temperature for 1 minute. After the slides were air-dried, 8 µl of mounting medium (Vectashield) was applied to each of the slides. FISH images were captured using Metasystems ISIS imaging system with sequential DAPI, spectral green and spectral orange filter setting.

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Results

Presenting history of decedent

The case of sudden unexpected nocturnal death of a 36 year old woman, was referred to the Cardiac Inherited Disease Group by the forensic pathologist for cardiac/genetic investigation. A thorough autopsy had revealed no cause for the death. Physical examination at autopsy was unremarkable, and in particular, no dysmorphic features were noted.

A review of clinical notes revealed that there had been a previous personal history of "drop attacks" followed by seizure, which had been investigated by a neurologist. She had had a normal electroencephalogram and cerebral CT scan. An electrocardiogram (ECG) obtained from the proband prior to a routine general anaesthetic for a tympanotomy performed four years prior to death was recovered from clinical records. The T waves were noted to be of low amplitude and late onset, with a prolonged QTc (Figure 1A). A discussion with the parents revealed no known family history of cardiac arrhythmia or sudden cardiac arrest.

Initial molecular genetic analysis

Molecular sequencing analysis of the genes associated with LQT syndrome types 1, 2, 3, 5, 6, 7 was performed on DNA extracted from an autopsy blood sample, [12] and no pathogenic mutations were detected. Given the appearance of the ECG, the decision was made to proceed with deletion/duplication analysis.

Deletion/duplication analysis

Array CGH analysis identified an approximately 542kb heterozygous deletion on chromosome 17 (17q24.3, hg19 coordinates chr17:67,714,894-68,256,981). This deletion encompasses two

human RefSeq genes, *KCNJ16* and *KCNJ2*; only the latter is recorded as a disease-causing gene in the Online Mendelian Inheritance in Man (OMIM) database (entry 600681; Figure 2).

Further clinical features

On identifying the mutation, the parents were re-interviewed and examined by a clinical geneticist. In addition, photographs of the decedent were examined for characteristic clinical features of ATS. She had no history of episodic flaccid muscle weakness or mild permanent symptomatic weakness and no dysmorphic features (such as low-set ears, widely spaced eyes, small mandible, syndactyly, short stature, or scoliosis). Furthermore, there was no evidence of mild learning difficulties or problems with executive function/ abstract reasoning.

Clinical history and examination of the proband's parents was likewise unremarkable. No abnormalities were seen on the father's ECG, but the mother's ECG showed aberrant repolarisation, prominent U-waves and a prolonged QT interval. In lead II, QT was 441msec, the preceding R-R was 915ms, giving a QTc 482msec, and in V5 QT was 474msec with preceding R-R of 983msec, giving a QTc of 478msec. The U wave became remarkably exaggerated after exercise (Figure 1B), but no ventricular arrhythmia was induced. These observations suggested that the proband's mother may carry the same intrachromosomal deletion identified in her daughter.

Parental analysis – FISH

FISH studies of the proband's parents revealed only a single signal for a probe encompassing the *KCNJ2* gene in the mother, confirming a heterozygous deletion of the same region as that identified in her daughter, while the father showed a pattern consistent with a normal copy number (Figure 3).

Discussion

Missense mutations are common within the *KCNJ2* gene,[13] with a mutational hotspot detected at Arg218.[14] Five small intragenic deletions (each less than 12bp in length) have been reported, four of which were associated with the typical clinical features of ATS.[15-17] Functional analysis of the consequence of a range of ATS mutations, including a small in-frame deletion, suggests that these mutations result in loss of function of the Kir2.1 channel.[16] The majority of these mutations have been shown to cause dominant-negative suppression of Kir2.1 channel current.[16-21] The underlying pathogenic mechanism is most frequently either defective trafficking or altered PIP_2-channel interactions, including less efficient binding. Sustained depolarisation of the muscle membrane and a failure to propagate action potentials is believed to cause the intermittent flaccid muscle paralysis that is a feature of ATS.[22]

It has become evident over the last five years that approximately 5% of cases of LQTS are due to intragenic deletions or duplications that are too large to be detected by routine sequence analysis (Table 1).[5-7] In terms of the *KCNJ2* gene, only two of 68 mutations reported in the HGMD Professional database (accessed 22.10.2013) are gross deletion or duplication/insertion mutations. With respect to the former, this patient carries a *de novo* 4Mbp heterozygous deletion (Figure 2) with a drug-induced prolonged QT interval, feeding difficulties, delayed dentition, hypertelorism, low set ears, micrognathia and hypotonia.[23] These authors suggested that many of these features overlapped with those found in ATS, which is characterised by periodic paralysis, ventricular arrhythmias, prolonged QT intervals, dental anomalies, small hands and feet, low-set ears, hypertelorism, micrognathia, fifth-digit clinodactyly and syndactyly, as well as joint laxity. Interestingly, other patients not reported in the HGMD Professional database have been described who carry deletions in the chromosome 17q24.2q24.3 region (Figure 2). The 2.3Mbp *de novo* heterozygous deletion carried by the

patient described by Blyth et al[24] exhibited posterior laryngeal cleft, delayed dentition, microcephaly, hypertelorism, bilateral 2/3-toe syndactyly, marked 5th finger clinodactyly and moderate mental retardation. The extent of this patient's deletion did not encompass the *KCNJ2* gene, and the authors suggested that reduced expression of the *KPNA2* gene may give rise to Nijemgen Breakage Syndrome that overlaps with some of their patient's dysmorphic features, while haploinsufficiency of PRKAR1A (known to cause Carney Complex) may account for other phenotypic features. Taken together, the dysmorphic features found in these two patients[23,24] may be due to gene expression effects that are not a consequence of copy number changes of the *KCNJ2* gene. Finally, Lestner et al[25] reported a *de novo* 2.5Mbp (maximum) heterozygous deletion encompassing the *KCNJ2* gene in a patient who presented after birth to a Genetics Department due to facial dysmorphisms, feeding difficulties and mild generalised hypotonia. Later clinical investigations identified skeletal malformations, but serial 12-lead ECGs showed no QT interval prolongation. These authors suggested that some of the dysmorphic features seen in their patient correlated with those reported in ATS; however, haploinsufficiency of a gene or genes further proximal may also play a role, such as the *MAP2K6* gene.

The proband reported here exhibits none of the dysmorphic features of ATS, and is unique in carrying a deletion that only encompasses the *KCNJ2* and *KCNJ16* gene. In terms of the latter, *Kcnj16*$^{(-,-)}$ mice exhibit no observable behavioural or physical abnormalities.[26] Critically, the findings of this present report support those of Limberg et al[27], who recently showed that *KCNJ2* gene mutations without a dominant negative effect on Kir2.x channels result in an isolated cardiac phenotype, without any of the typical dysmorphic, skeletal muscular or neurocognitive features typical of ATS. Usually, individuals with ATS are considered to be at

lower risk of sudden cardiac death than for other LQT syndromes.[14] However 50% of affected individuals appear to carry a *de novo* mutation, the condition is very rare, and the pure cardiac phenotype is a minority[13] such that comparisons with the more common forms of long QT syndrome are difficult.

The heterozygous deletion of the *KCNJ2* gene in our patient and her mother would be expected to lead to haploinsufficiency of the Kir2.1 channel, with none of the dominant-negative effects seen with other mutations. However, a reduction in Kir2.1 to ~50% could still lead to the generation of spontaneous ventricular activity and the attendant risk of a lethal ventricular arrhythmia, particularly in the presence of environmental or physiological triggers.

To the best of our knowledge, there have been no previous reports of any large deletion mutations detected first by molecular autopsy. Since large gene re-arrangements seem to have a tendency to produce a more severe phenotype,[5] further study of their prevalence among sudden unexplained death victims would seem worthwhile.

Array Comparative Genomic Hybridization and FISH

Our approach to screen multiple genes simultaneously for deletion and duplication mutations has been to use aCGH technology. To our knowledge this has not been applied to any area of inherited heart disease to date. The array we have used targets a select list of genes implicated in heritable cardiac disorders, but also allows for low resolution whole genome screening. The outcome of our analysis has been to identify the heterozygous loss of the *KCNJ2* gene, but also the approximate extent of the deletion that encompasses this gene. This approach therefore offers greater resolution and versatility compared to the more conventional MLPA method that targets defined regions in most exons, but sometimes not all, of a small number of genes.

As a consequence of the aCGH approach, FISH could be used to determine if the deletion event in the decedent were *de novo*. FISH is a standard cytogenetic tool that allows for targeted dosage analysis to be undertaken, but is limited to regions of the genome for which there are relevant probes. The probes that are used are largely cloned DNAs whose positions in the human genome are known, but must be large enough to provide a detectable signal. The latter, in turn, requires that the dosage event being screened is large enough so as to encompass the probe. These conditions were satisfied in the case reported here.

Conclusions

The case we describe here is, we believe, the first report of a whole gene heterozygous deletion as a cause of sudden cardiac death or of long QT syndrome. It is also the first to report the use of aCGH to discover mutations in genes linked to sudden cardiac death, indeed for any inherited heart condition. The extent of the deletion event identified in the decedent allowed the use of FISH to determine if the event were *de novo*, which is a cheaper method compared to aCGH. Finally, since there were none of the systemic features of ATS despite a whole gene deletion of KCNJ2 in two subjects with long QT syndrome, this report supports recent research suggesting that a dominant negative effect may be essential for this full syndrome to occur.

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Conflicts of Interests Disclosures: None.

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Table 1 Mutation load in LQT genes (accessed HGMD Professional database 23.10.2013)

Type	OMIM	Genes	Refseq Accession Number	Gross deletions	Gross insertions/ duplications	Complex rearrangements	Total number of mutations	% of all mutations that are deletions/duplications/complex re-arrangements
LQT1	192500	*KCNQ1*	NM_000218.2	9	1	5	466	3.2%
LQT2	152427	*KCNH2* *(HERG)*	NM_000238.3	13	6	5	687	3.5%
LQT3	603830	*SCN5A* *ANK2*	NM_198056.2	1	0	1	660	0.3%
LQT4	600919	*(LQT4)*	NM_001148.4	0	0	0	18	0.0%
LQT5	176261	*KCNE1*	NM_000219.4	0	0	0	40	0.0%
LQT6	603796	*KCNE2*	NM_172201.1	0	0	0	20	0.0%
LQT7	170390	*KCNJ2*	NM_000891.2	1	1	0	68	2.9%
LQT8	601005	*CACNA1C* *CAV3*	NM_000719.6	0	0	0	23	0.0%
LQT9	611818	*(Caveolin 3)*	NM_033337.2	1	0	0	40	2.5%
LQT10	611819	*SCN4B*	NM_174934.3	0	0	0	5	0.0%
LQT11	611820	*AKAP9*	NM_005751.4	0	0	0	4	0.0%
LQT12	601017	*SNTA1*	NM_003098.2	0	0	0	7	0.0%
LQT13	600734	*KCNJ5* *(GIRK4)*	NM_000890.3	0	0	0	5	0.0%

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Figure Legends:

Figure 1 - Electrocardiograms of proband and her mother. **Panel A:** 12 lead electrocardiogram recorded from the proband four years prior to her death. The T waves are of low amplitude and have a late onset. The automated measurement calculates a QTc of 474msec. Direct measurement with digital callipers in lead II gives QT 513msec, preceding R-R 1100msec; QTc 489msec. Lead V5 QT 489msec, R-R 1100msec, QTc 506msec. **Panel B:** 12 lead electrocardiogram recorded from the mother of proband post exercise, during family screening at age 66 years. The repolarisation looks abnormal, with the U wave of similar or greater amplitude than the T wave. In lead II, QT is 330msec, preceding R-R 705ms, giving QTc 393msec, but to the end of the T-U complex is 522msec giving QTUc 622ms. In V5, QT is 330msec with preceding R-R 691msec, QTc 397msec and QU is529msec, giving QTUc 636ms.

Figure 2 - Schematics of proband's chromosome 17 heterozygous deletion and other microdeletions. **Panel A:** DEVA software output showing a copy number change (deletion; 198 probes; log2ratio: -0.5459) localized to 17q24.3(67,714,894-68,256,981)x1 (hg19 co-ordinates). **Panel B:** Ideogram of chromosome 17 showing the location and extent of microdeletions reported in the literature and the proband reported here (hg19 co-ordinates). BAC probes used in the FISH studies, as well genes that are localised to chromosome 17q24.2q24.3, and those that are reported as disease-causing in the OMIM database, are shown. These graphics were largely taken from the UCSC genome browser (http://genome.ucsc.edu/).

Figure 3 - FISH analysis of parents' chromosomes**. Panel A:** the mother's chromosomes show a

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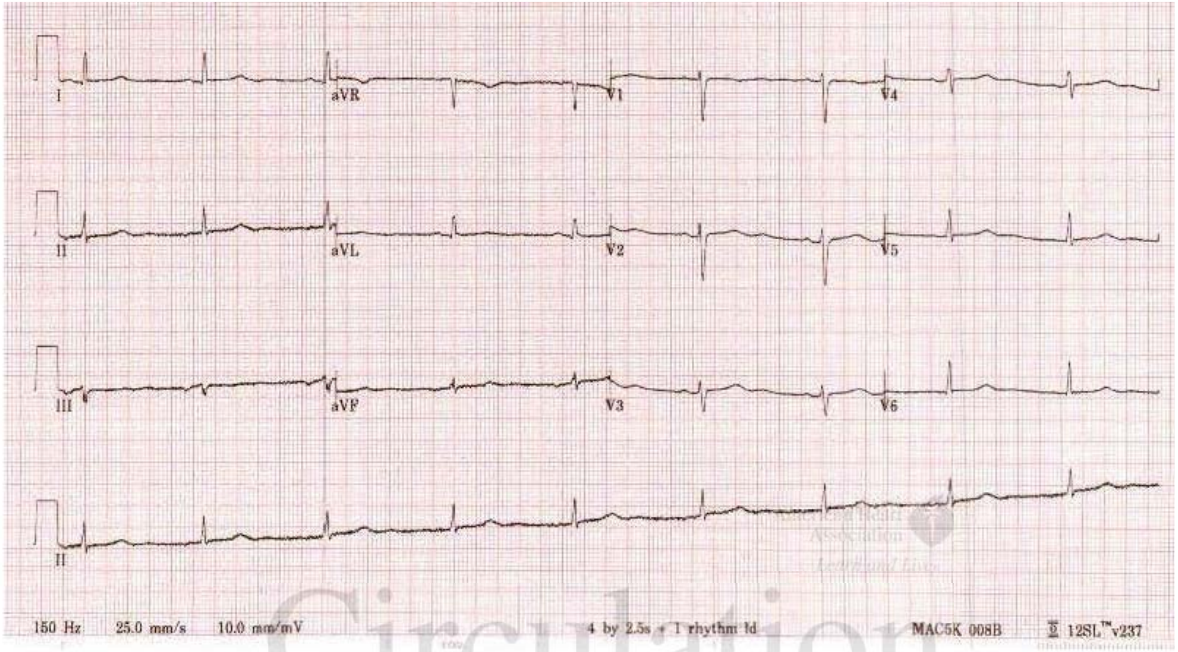
loss of one orange signal (RP11-633A13 and RP11-693H11) indicating a heterozygous deletion. **Panel B:** the father's chromosomes show no deletion of the region encompassed by RP11-633A13.

A



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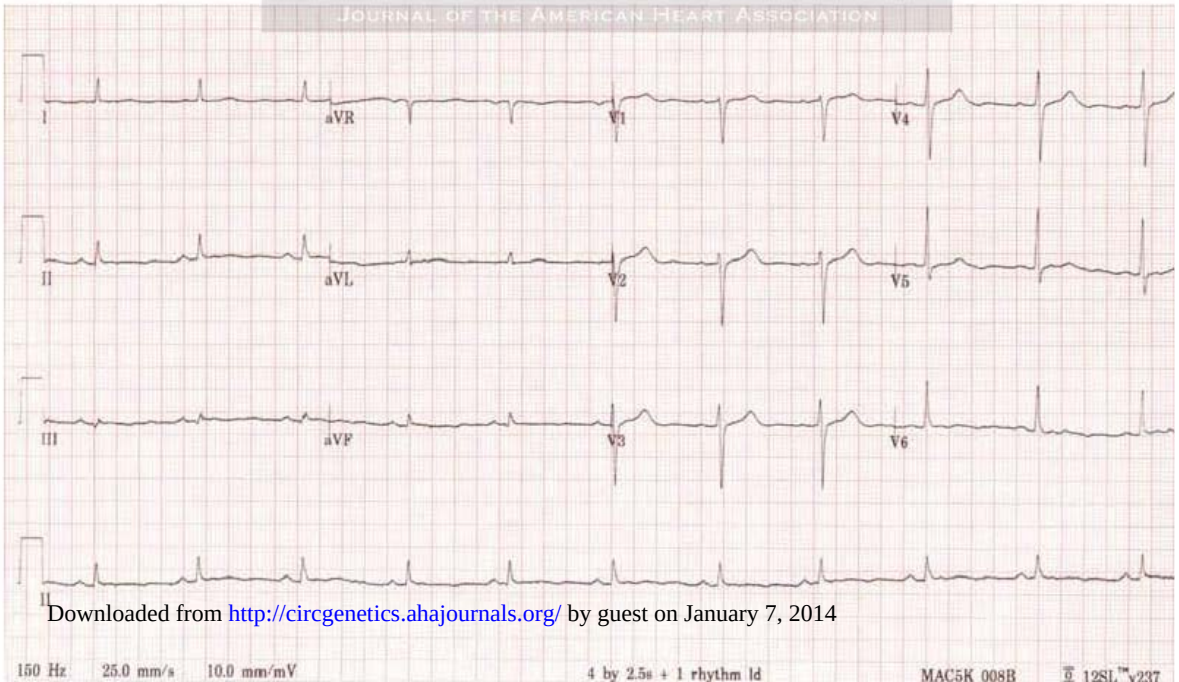

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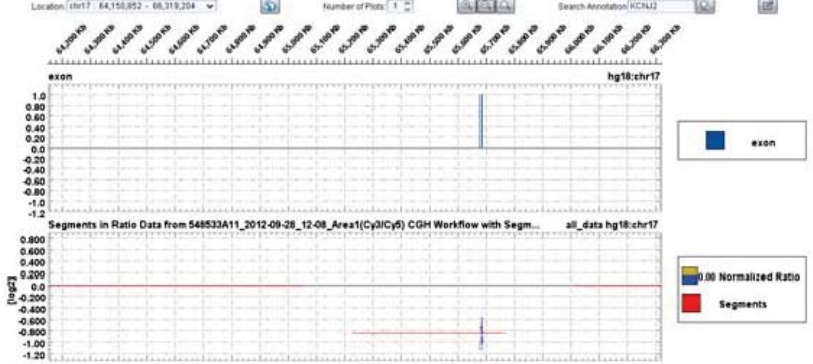
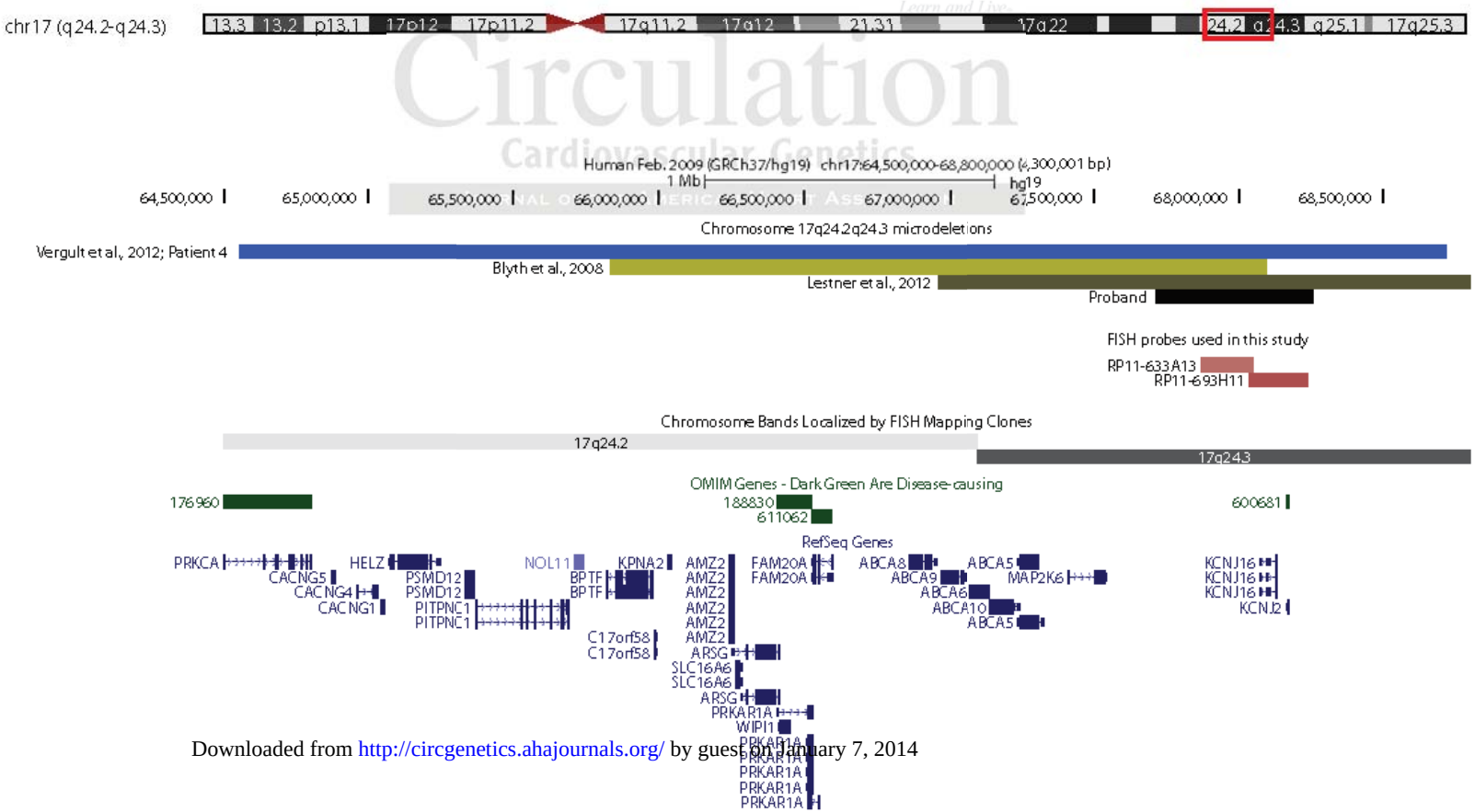


B



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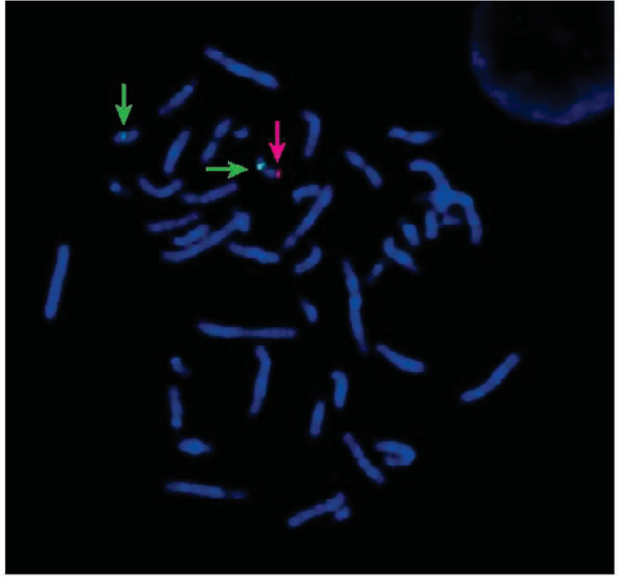

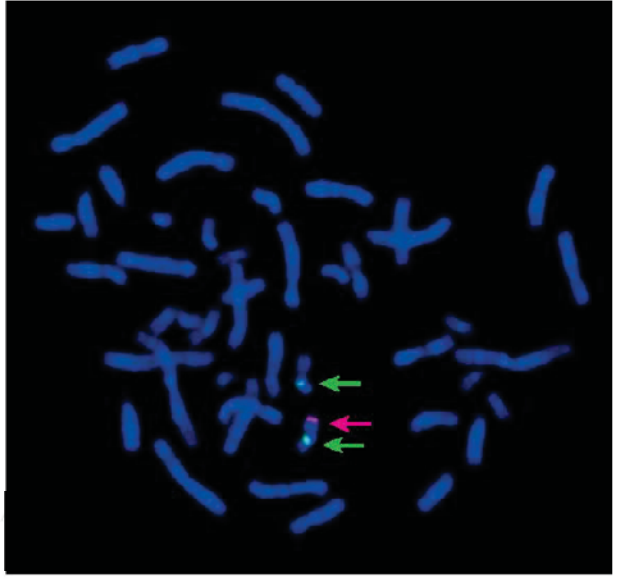

RP11-633A13

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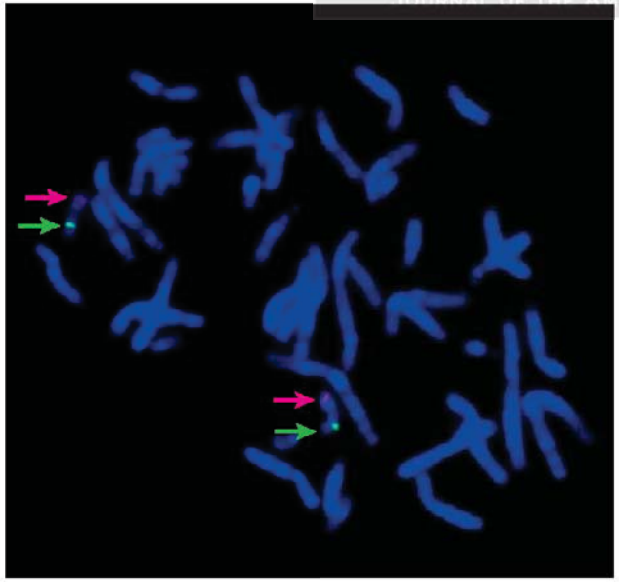

RP11-633A13

SUPPLEMENTAL MATERIAL

Full list of genes targeted by 12x135K Custom CGH array (Roche NimbleGen)

Gene name	Description	mRNA Accession number	OMIM ID
KCNQ1	Potassium voltage-gated channel subfamily KQT; LQT1	NM_000218.2	607542
KCNH2	Potassium voltage-gated channel subfamily H; LQT2	NM_000238.2	152427
SCN5A	Sodium channel protein type 5 subunit alpha; LQT3/BRUGADA	NM_198056.2	600163
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like	NM_015141.3	611778
SCN1B	Sodium channel subunit beta-1 isoform a	NM_001037.4	600235
SCN3B	Sodium channel subunit beta-3 precursor	NM_018400.3	608214
CACNB2	Voltage-dependent L-type calcium channel subunit	NM_201596.2	600003
KCNE3	Potassium voltage-gated channel subfamily E	NM_005472.4	604433
ANK2	Ankyrin-2 isoform 1; LQT4	NM_001148.4	106410
KCNE1	Potassium voltage-gated channel subfamily E; LQT5	NM_000219.3	176261
KCNE2	Potassium voltage-gated channel subfamily E; LQT6	NM_172201.1	603796
KCNJ2	Inward rectifier potassium channel 2; LQT7/ATS	NM_000891.2	600681
CACNA1c	Voltage-dependent L-type calcium channel subunit; LQT8	NM_001129827.1	114205
CAV3	Caveolin-3; LQT9	NM_033337.2	601253
SCN4B	Sodium channel subunit beta-4 isoform 1; LQT10	NM_174934.3	608256
AKAP9	A-kinase anchor protein 9 isoform 2; LQT11	NM_005751.4	604001
CACNA2D1	Voltage-dependent calcium channel subunit	NM_000722.2	114204
KCND3	Potassium voltage-gated channel subfamily D	NM_004980.4	605411
KCNE3	Potassium voltage-gated channel subfamily E	NM_005472.4	604433
SNTA1	Alpha-1-syntrophin; LQT12	NM_003098.2	601017
SYNE1	Nesprin-1 isoform 1	NM_182961.2	608441
MYH7	Myosin, heavy chain 7	NM_000257.2	160760
MYBPC3	Myosin-binding protein C, cardiac-type	NM_000256.3	600958
TNNT2	Troponin T, cardiac muscle isoform 1	NM_000364.2	191045
TNNI3	Troponin I, cardiac muscle	NM_000363.4	191044
TPM1	Tropomyosin alpha-1 chain isoform 7	NM_001018020.1	191010
ACTC1	Actin, alpha, cardiac muscle 1	NM_005159.4	102540
MYL2	Myosin regulatory light chain	NM_000432.3	160781

	2		
MYL3	Myosin light chain 3	NM_000258.2	160790
LAMP2	Lysosome-associated membrane glycoprotein 2	NM_001122606.1	309060
PRKAG2	5'-AMP-activated protein kinase subunit gamma-2	NM_016203.3	602743
CSRP3	Cysteine and glycine-rich protein 3	NM_003476.3	600824
TCAP	Telethonin	NM_003673.3	604488
PLN	Cardiac phospholamban	NM_002667.3	172405
GLA	Alpha-galactosidase A precursor	NM_000169.2	300644
RYR2	Ryanodine receptor 2; CPVT1	NM_001035.2	180902
CASQ2	Calsequestrin-2 precursor; CPVT2	NM_001232.3	114251
DSP	Desmoplakin isoform I	NM_004415.2	125647
PKP2	Plakophilin-2 isoform 2a	NM_001005242.2	602861
DSG2	Desmoglein-2 preproprotein	NM_001943.3	125671
DSC2	Desmocollin-2 isoform Dsc2a preproprotein	NM_024422.3	125645
JUP	Junction plakoglobin	NM_002230.2	173325
TMEM43	Transmembrane protein 43	NM_024334.2	612048
TGFB3	Transforming growth factor beta-3 preproprotein	NM_003239.2	190230
DES	Desmin	NM_001927.3	125660
KCNA1	Potassium voltage-gated channel, shaker-related subfamily, member 1	NM_000217.2	176260
FBN1	Fibrillin-1 precursor	NM_000138.4	134797
FBN2	Fibrillin-2 precursor	NM_001999.3	612570
TGFBR2	TGF-beta receptor type-2 isoform A precursor	NM_001024847.2	190182
CACNA1B	voltage-dependent N-type calcium channel subunit	NM_000718.2	601012
NPPA	Atrial natriuretic factor preproprotein	NM_006172.3	108780
KCNA5	Potassium voltage-gated channel subfamily A	NM_002234.2	176267
PSEN1	Presenilin-1 isoform I-467	NM_000021.3	104311
PSEN2	Presenilin-2 isoform 1	NM_000447.2	600759
TTID	Myotilin isoform a; LGMD-1A	NM_006790.2	604103
LMNA	Lamin A/C isoform 1 precursor; LGMD-1B	NM_170707.2	150330
CAV3	Caveolin 3; LGMD-1C	NM_033337.2	601253
CAPN3	Calpain 3 isoform a; LGMD-2A	NM_000070.2	114240
DYSF	Dysferlin isoform 1; LGMD-2B	NM_001130987.1	603009
SGCG	Gamma sarcoglycan; LGMD-2C	NM_000231.2	608896

SGCA	Sarcoglycan, alpha isoform 1 precursor; LGMD-2D	NM_000023.2	600119
SGCB	Sarcoglycan, beta; LGMD-2E	NM_000232.4	600900
SGCD	Delta-sarcoglycan isoform 1; LGMD-2F	NM_000337.5	601411
TCAP	Telethonin; LGMD-2G	NM_003673.3	604488
TRIM32	TAT-interactive protein, 72-KD; LGMD-2H	NM_012210.3	602290
FKRP	Fukutin-related protein; LGMD-2I	NM_024301.4	606596
TTN	Titin isoform N2-A; LGMD-2J	NM_133378.4	188840
POMT1	Protein O-mannosyl-transferase 1 isoform a; LGMD-2K	NM_007171.3	607423
ANO5	Anoctamin-5 isoform a; LGMD-2L	NM_213599.2	608662
FKTN	Fukutin isoform a; LGMD-2M	NM_001079802.1	607440
POMT2	Protein O-mannosyl-transferase 2; LGMD-2N	NM_013382.5	607439
GAA	Lysosomal alpha-glucosidase preproprotein; GSD II	NM_000152.3	606800
VCP	Transitional endoplasmic reticulum ATPase	NM_007126.3	601023
LAMA2	Laminin subunit alpha-2 isoform a precursor	NM_000426.3	156225
POMGNT1	Protein O-linked mannose beta1,2-N-acetylglucosaminyltransferase, transcript variant 1; MEB	NM_017739.3	606822
SEPN1	Selenoprotein N isoform 1 precursor; RSMD1	NM_020451.2	606210
ITGA7	Integrin alpha-7 isoform 1 precursor	NM_001144996.1	600536
DES	Desmin	NM_001927.3	125660
DAG1	Dystroglycan 1	NM_001165928.2	128239
SLC25A4	ADP/ATP translocase 1	NM_001151.3	103220
MYOZ2	Myozenin-2	NM_016599.3	605602
MYLK2	Myosin light chain kinase 2, skeletal/cardiac	NM_033118.3	606566
JPH2	Junctophilin-2 isoform 1	NM_020433.4	605267
LDB3	LIM domain-binding protein 3 isoform 1	NM_007078.2	605906
ABCC9	ATP-binding cassette sub-family C member 9	NM_020297.2	601439
VCL	Vinculin isoform VCL	NM_014000.2	193065
TNNC1	Troponin C, slow skeletal and cardiac muscles	NM_003280.2	191040
ACTN2	Alpha-actinin-2	NM_001103.2	102573
NEXN	Nexilin isoform 1	NM_144573.3	613121
RBM20	Probable RNA-binding protein 20	NM_001134363.1	613171
MYH6	Myosin-6	NM_002471.3	160710

TAZ	Tafazzin isoform 1	NM_000116.3	300394
DTNA	Dystrobrevin alpha isoform 1	NM_001390.4	601239
EYA4	Eyes absent homolog 4 isoform a	NM_004100.4	603550
TMPO	Thymopoietin isoform alpha	NM_003276.2	188380
GJA5	Gap junction alpha-5 protein	NM_005266.5	121013
ELN	Elastin isoform a precursor	NM_000501.2	130160
CTF1	Cardiotrophin-1 isoform 1	NM_001330.3	600435
EMD	Emerin	NM_000117.2	300384
TTR	Transthyretin precursor	NM_000371.3	176300
LAMP2	Lysosome-associated membrane glycoprotein 2	NM_002294.2	309060