Triadin Knockout Syndrome is Absent in a Multi-Center Molecular Autopsy Cohort of Sudden Infant Death Syndrome and Sudden Unexplained Death in the Young and is Extremely Rare in the General Population

Running title: *Clemens et al.; Prevalence of Triadin Knockout Syndrome*

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Abstract:

Background - Triadin knockout syndrome (TKOS) is a potentially lethal arrhythmia disorder caused by recessively inherited null variants in *TRDN*-encoded cardiac triadin. Despite its malignant phenotype, the prevalence of TKOS in sudden infant death syndrome (SIDS) and sudden unexplained death in the young (SUDY) is unknown.

Methods - Exome sequencing was performed on 599 SIDS and 258 SUDY cases. Allele frequencies of all *TRDN*-null variants identified in the cardiac specific isoform of *TRDN* in the Genome Aggregation Database (gnomAD) were used to determine the estimated prevalence and ethnic distribution of TKOS.

Results - No triadin null individuals were identified in 599 SIDS and 258 SUDY exomes. Using gnomAD, we estimate the overall prevalence of TKOS to be ~1:22.7 million individuals. However, TKOS prevalence is 5.5-fold higher in those of African descent (~1:4.1 million).

Conclusions - TKOS is an exceedingly rare clinical entity that does not contribute meaningfully to either SIDS or SUDY. However, despite its rarity and absence in large sudden death cohorts, TKOS remains a malignant and potentially lethal disorder which requires further research in order to better care for these patients.

Key words: arrhythmia; genetics; pediatrics; sudden death; Triadin

Nonstandard Abbreviations and Acronyms

CPVT	catecholaminergic polymorphic ventricular tachycardia
CRU	calcium release unit
gnomAD	Genome Aggregation Database
ITKOSR	International Triadin Knockout Syndrome Registry
LoF	loss-of-function
LQTS	long QT syndrome
oe	observed/expected score
pLI	probability of being loss-of-function
RyR2	*RYR2*-encoded ryanodine receptor/calcium release channel
SIDS	sudden infant death sydnrome
SUDY	sudden unexplained death in the young
TKOS	triadin knockout syndrome

Introduction

Sudden unexplained death is defined as a sudden and unexpected death that remains unexplained after a thorough clinical history, death scene investigation, and postmortem examination. These cases can be categorized into two main groups including sudden infant death syndrome (SIDS) which is defined as sudden unexplained death in an infant under the age of 1 year, and accounts for approximately 3000 sudden deaths each year in the United States (US), and sudden unexplained death in the young (SUDY) which typically includes those between the ages of 1 and 35 and occurs in up to 5000 individuals in the US each year.[1] Previous studies have shown that ~5-10% of SIDS and ~25-30% of SUDY are caused by variants in genetic heart disease-susceptibility genes, particularly those which underlie the channelopathies and cardiomyopathies. The most highly represented diseases in these studies are often long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT).[2-4]

Since 2012, mutations in *TRDN*-encoded cardiac triadin have been implicated as an underlying cause in each of these arrhythmia syndromes.[5-9] Interestingly, these patients display clinical features of multiple arrhythmia syndromes including transient QT prolongation, ventricular ectopy upon stress testing, and extensive T-wave inversion in precordial leads V1-V4. This suggests that these patients do not have typical LQTS or CPVT, but instead suffer from a distinct overlap disorder which is now referred to as triadin knockout syndrome (TKOS).[10]

TKOS is a potentially lethal recessively inherited arrhythmia syndrome caused by homozygous or compound heterozygous null mutations in *TRDN*. Recently published data from the International Triadin Knockout Syndrome Registry (ITKOSR) found that 95% of TKOS patients are symptomatic and present at an average age of 3 years with all patients presenting by 10 years of age. Additionally, 81% of patients have suffered at least 1 cardiac arrest and 10%

experienced sudden cardiac death.[10] Individuals who are heterozygous for only a single *TRDN* null allele are unaffected.

Despite its malignant and potentially lethal phenotype, the prevalence of TKOS in cases of sudden unexplained death remains unknown. Therefore, we sought to determine the prevalence of TKOS in a large multi-center molecular autopsy cohort of SIDS and SUDY cases as well as its prevalence and ethnic distribution in the general population.

Methods

In order to prevent the re-identification of individuals included in this study, individual patient data will not be made available to other researchers. This study complies with the Declaration of Helsinki; locally appointed ethics committees including Mayo Clinic's Institutional Review Board have approved the research protocol. Data from the Genome Aggregation Database (gnomAD) is publically available at https://gnomad.broadinstitute.org/.[11] The detailed methods are included in the Supplemental Material.

Results

Demographics

We analyzed 857 sudden unexplained death cases. Of these, 599 were SIDS and 258 were SUDY (**Table 1**). Of the SIDS cases, 362 (60%) were male and 237 (40%) were female. The median age at death was 2 months (interquartile range (IQR) 1-4 months), and 349 (58%) of these cases were white. In the SUDY cohort, 176 (68%) cases were male and 82 (32%) were female. The median age at death was 21 years (IQR 16-29 years) with the vast majority dying between the ages of 1-35 years (88%), and 196 (76%) were white (**Table 1**).

Prevalence of TKOS in SIDS and SUDY

Following next-generation sequencing and gene specific analysis of *TRDN*, none of the 599 SIDS cases or 258 SUDY cases harbored either homozygous or compound heterozygous *TRDN* null variants. In fact, none of the SIDS or SUDY cases hosted even a single *TRDN* null allele (**Supplemental Figure 1**). We also assessed all cases for the presence of any novel homozygous or compound heterozygous missense variants which could potentially be disease causing, but none were identified. Additionally, despite the absence of TKOS in our sudden death cohorts, we were able to utilize an exact binomial confidence interval (CI) to calculate an upper bound of the frequency of TKOS in sudden death, and this was determined to be 0.43% (95% CI = 0, 0.0043).

Estimated Prevalence and Ethnic Distribution of TKOS

In gnomAD (n=141,456 individuals) a total of 39 loss-of-function (LoF) variants were observed in *TRDN* compared to the expected number of 50.1 LoF variants leading to a probability of being loss-of-function intolerant (pLI) score of 0 and an observed/expected (oe) score of 0.78 (90% CI = 0.6-1.02). This would indicate that *TRDN* is extremely tolerant of LoF variation. However, this is to be expected as *TRDN* haploinsufficient individuals do not display a disease phenotype and only individuals with recessively inherited homozygous/compound heterozygous null variants have TKOS.

In order to estimate the prevalence of TKOS in the general population, we identified all cardiac specific isoform *TRDN* null variants present in gnomAD (**Supplemental Figure 2**). Overall, we identified only 16 unique *TRDN* null variants (**Figure 1; Table 2**). Of these, 7 (44%) were frameshift, 4 (25%) splice-altering, 3 (19%) nonsense, and 2 (12%) missense. Importantly, none of the null variants were present as homozygous in gnomAD. Although we are unable to exclude the possibility that an individual in gnomAD could be compound heterozygous for

TRDN null variants, individuals known to be affected by severe pediatric disease have been removed from the gnomAD data set making it highly unlikely that any individuals in gnomAD have TKOS.[11] Six of the gnomAD *TRDN* null variants (N9fs*5, D18fs*14, T59M, W60L, K147*0, and Q205*) have been identified previously in cases of TKOS (**Figure 1; Table 2**).[5-8, 10, 12] The majority of variants (9/16; 56%) were observed only one time in gnomAD and 7 variants (44%) were present in greater than one allele. The D18fs*14 variant was the most common null variant with a gnomAD allele account of 13 times in 280,480 total alleles. The total allele account for all 16 identified variants was 43 (**Supplemental Figure 2; Table 2**).

However, the sequencing coverage of *TRDN* in gnomAD is poor. In fact, only 3 of the 8 exons (exons 1, 2, and 8) that make up the cardiac specific isoform of *TRDN* have greater than 30X coverage. The other 5 exons all have coverages of ~20X or less. This causes variability in the number of successfully sequenced alleles at each variant location. For example, the D18fs*14 variant was observed 13 times in 280,480 alleles, but K147fs*0 was present in 2 out of only 89,152 alleles (**Table 2**). In order to correct for this suboptimal coverage and variable allele number across variants, we multiplied the allele frequency of each variant by the total number of alleles in gnomAD to generate an adjusted allele count (**Supplemental Figure 2)**. Following these calculations, the estimated total adjusted allele count for the 16 *TRDN* null variants in gnomAD was 59.38 (**Table 2**).

We then used the total adjusted allele count to calculate the frequency of both *TRDN* null variants and triadin null individuals in the population. By dividing the total adjusted allele count by the total individuals in gnomAD, we estimate that 1:2,382 individuals in gnomAD have a single *TRDN* null variant (**Supplemental Figure 2; Table 3**). Furthermore, by utilizing the Hardy-Weinberg equation, we estimate that ~1:22.7 million people in the general population

may have TKOS stemming from the presence of two *TRDN* null alleles (**Supplemental Figure 2; Table 3**).

In addition, we analyzed the prevalence of triadin null status in each of the 8 ethnic populations represented in gnomAD. Five of the 8 ethnic populations had a *TRDN* null allele count ≥ 1 (**Table 2**). The most highly represented group in gnomAD was Europeans who had an estimated total adjusted allele count of 34.22 and an estimated triadin null frequency of ~1:14.3 million. The ethnicity with the highest frequency of triadin null individuals was African with a total adjusted allele count of 12.39 and an estimated frequency of ~1:4.1 million. Interestingly, all 12 unrelated African individuals in gnomAD with a *TRDN* null variant had the same D18fs*14 variant (**Tables 2 and 3**). Three of the populations (Ashkenazi Jewish, European [Finnish], and "other") had allele counts of 0. Therefore, we were unable to determine the estimated frequency of triadin null individuals in these populations (**Tables 2 and 3**).

Discussion

TRDN-encoded cardiac triadin is a critical protein within the cardiac calcium release unit (CRU) complex where the L-type calcium channel is juxtaposed to the *RYR2*-encoded ryanodine receptor/calcium release channel (RyR2) on the junctional sarcoplasmic reticulum.[13, 14] This multi-protein complex is responsible for mediating calcium sensing and proper excitation-contraction coupling in the heart. Triadin binds to multiple proteins within the CRU including RyR2, calsequestrin 2, and junctin and helps mediate proper sarcoplasmic reticulum calcium release through RyR2, as well as stabilization of the CRU structure.[14, 15]

Loss of triadin due to homozygous or compound heterozygous null mutations causes a distinct overlap arrhythmia disorder known as triadin knockout syndrome (TKOS).[6, 10] Patients

with TKOS typically exhibit clinical features of multiple arrhythmia syndromes including QT prolongation, ventricular ectopy upon stress testing, and T-wave inversion in the precordial leads. TKOS also manifests with a particularly severe phenotype including sudden cardiac arrest at a young age, and recurrent breakthrough cardiac events despite various treatment strategies. Considering this unique set of features which are common among these patients, there is a strong likelihood that future patients who fit this phenotypic profile may have TKOS.

Additionally, 2/21 known TKOS patients have died following sudden cardiac arrest.[10] Therefore, in this study we sought to determine the prevalence of TKOS in a large, multi-center cohort of sudden unexplained death cases. Interestingly, we were not able to identify any triadin null individuals in our 599 SIDS or 258 SUDY cases. This has led us to conclude that, despite its malignant and potentially lethal phenotype, TKOS does not seem to contribute meaningfully to either SIDS or SUDY, at least among Caucasian decedents.

Here, we examine three viable explanations for the apparent absence of TKOS in sudden death involving infants, children, adolescents, and young adults. The most likely explanation for the absence of TKOS in our sudden death cohorts is simply the extreme rarity of the disease. According to our gnomAD-derived prevalence estimates, triadin null status may be as rare as ~1:22.7 million individuals in the general population. Therefore, despite the relatively large size of our sudden death cohorts (compared to most molecular autopsy studies), we remain underpowered to capture a case of TKOS. It should be noted that these estimates are for triadin null individuals, and not necessarily for TKOS. While TKOS is currently thought to be extremely penetrant (95%)[10], it remains to be seen whether mild cases will emerge as has been observed for most other arrhythmia syndromes.[16]

One of the main issues we encountered by using gnomAD for our prevalence estimates was the poor and variable sequencing coverage of *TRDN* which suggests that this is a difficult gene to sequence successfully. We were able to correct for poor coverage and generate an adjusted allele count for each *TRDN* null variant in gnomAD. However, null variants could have been missed in gnomAD due to poor sequencing coverage, and possibility remains that triadin null status, while still very rare, could be more common than our current estimates predict. Additionally, we recently performed whole exome sequencing and a nucleotide-level coverage analysis on 28 SUDY cases and their parents.[17] This analysis also showed poor sequencing coverage of multiple exons in *TRDN*. Therefore, physicians and investigators should consider coverage when performing genetic testing or whole exome/genome sequencing on a potential TKOS patient as low coverage could lead disease-causing variants to be missed.

A second potential explanation for this finding is age at death. As noted in the ITKOSR, all symptomatic patients presented between the ages of 1-10 years.[10] In addition to SIDS being a highly heterogeneous disorder caused by a variety of different issues, the observation that patients with TKOS do not seem to present until after their first birthday may provide an explanation for why we did not observe any triadin null cases in our SIDS cohort. We also assessed the distribution of ages within our SUDY cohort and found that only 32 cases, or about 12%, fell between the ages of 1 and 10, likely limiting our ability to capture any triadin knockout cases in this cohort.

A final reason *TRDN* null variants may be absent from our cohorts is ethnicity. While the frequency of triadin null individuals in the general population is estimated to be ~1:22.7 million, this frequency varies greatly across the different ethnic populations in gnomAD. The population in which it is predicted to be the most prevalent is in those of African descent (~1:4.1 million).

However, when we assessed the demographics of our SUDY cohort, we found that only ~7% of individuals were black, again severely limiting our ability to capture a triadin null individual in this study.

Interestingly, some TKOS-causative variants seem to be ethnic-specific. The most striking example of this is the D18fs*14-TRDN variant. Of the 13 individuals in gnomAD that host this variant, 12 of them are of African descent, and there are no African individuals in gnomAD that have any other *TRDN* null variants. Additionally, all of the patients in the ITKOSR, who have this variant, inherited it from a black parent.[10] Considering the strong correlation of this variant to individuals of African descent, it is probable that D18fs*14 is a founder variant in the black population. Another example of this is Q205*-TRDN. In gnomAD, there are 5 individuals with this variant, and 4 of them are European. This correlates with the registry data where we identified 5 patients with this variant, all of whom are white/European.[10]

Considering that the current world population is ~7.7 billion people, we predict based on gnomAD frequency that there may be 340 cases of TKOS worldwide. However, gnomAD is not necessarily an accurate representation of the world population, and ethnicity needs to be considered. In places where census data are available, such as the United States, we can utilize ethnic specific prevalence estimates to make these predictions. Using 2018 population estimates from the United States Census Bureau, we predict that there may be as many 28 cases of TKOS in the US. Of these, 14 would be white, 11 black, and 3 Latino. However, the number of TKOS cases is likely to be much higher in areas of the world where the majority of the population is black such as central Africa. In addition, because TKOS is a recessive disorder, we would expect the frequency of TKOS cases to be higher in the Middle East, South Asia, and North Africa where consanguineous marriages may constitute 20% to 50% of all marriages.[18] Interestingly, 6

of the 16 families in the ITKOSR are either Indian or Arabic, and in two of these families, the parents are confirmed to be consanguineous first- or second-degree cousins.

Limitations

There are a few minor limitations to this study. First, *TRDN* has poor exome sequencing coverage across the majority of exons in gnomAD and it is possible that null variants were missed. Second, we are unable to determine if an individual in gnomAD is compound heterozygous for *TRDN* null variants. However, this is unlikely due to exclusion of individuals with severe pediatric disease from gnomAD. Third, we only included missense variants known to render a null allele. Finally, while copy number variants have been shown to cause TKOS in a previously published patient, copy number variants were not included in our prevalence estimates.

Conclusions

Despite its rarity and absence in large sudden death cohorts, TKOS remains a malignant and potentially lethal disorder akin to other rare disorders such as calmodulinopathy and Timothy syndrome.[19, 20] Not only do TKOS patients present with life-threatening cardiac events at a very young age, but almost 75% of patients have experienced breakthrough cardiac events while on conventional therapies.[10] Therefore, the severe and treatment refractory nature of the disease highlights the urgent need for further research and development of novel treatment methods which are necessary in order to better care for these patients.

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Table 1. Demographics of the SIDS and SUDY Cohorts

	SIDS	**SUDY**
Total Cases	599	258
Sex		
Male	362 (60)	176 (68)
Female	237 (40)	82 (32)
Age at Death		
Average	2 (1-4) months	21 (16-29) years
Range	0-11 months	1-59 years
Ethnicity		
White	349 (58)	196 (76)

*Values are n (%) or median (interquartile range)

Table 2. *TRDN* Null Variants in GnomAD and Their Corresponding Allele Counts

		Allele Count / Adjusted Allele Count								
Triadin Null Variants (cardiac isoform)	**Total Allele Number**	**All**	**European (non-Finnish)**	**African**	**Latino**	**Ashkenazi Jewish**	**East Asian**	**European (Finnish)**	**South Asian**	**Other**
c. 22+1G>T	247,980	1 / 1.14	1 / 1.15	-	-	-	-	-	-	-
#c. 22+29A>G	247,606	1 / 1.14	1 / 1.15	-	-	-	-	-	-	-
#p. D18fs*14	280,480	13 / 13.11	-	12 / 12.39	1 / 1.00	-	-	-	-	-
p. K20fs*2	249,126	2 / 2.27	2 / 2.29	-	-	-	-	-	-	-
#p. T59M	249,154	4 / 4.54	2 / 2.29	-	2 / 2.05	-	-	-	-	-
#p. W60L	249,140	1 / 1.14	1 / 1.14	-	-	-	-	-	-	-
c. 424+2T>C	111,664	1 / 2.53	1 / 2.68	-	-	-	-	-	-	-
#p. K147*0	89,152	2 / 6.35	2 / 6.95	-	-	-	-	-	-	-
c. 485-2A>G	137,668	2 / 4.11	-	-	-	-	-	-	2 / 3.13	-
p. E176fs*47	147,340	1 / 1.92	1 / 2.30	-	-	-	-	-	-	-
p. L201fs*19	221,160	6 / 7.68	3 / 4.03	-	3 / 3.93	-	-	-	-	-
#p. Q205*	163,614	5 / 8.65	4 / 7.85	-	1 / 1.46	-	-	-	-	-
p. E221*	214,208	1 / 1.32	-	-	-	-	-	-	1 / 1.10	-
p. Q227fs*35	233,610	1 / 1.21	1 / 1.25	-	-	-	-	-	-	-
p. E249fs*16	248,788	1 / 1.14	-	-	-	-	1 / 1.11	-	-	-
p. E251*	248,790	1 / 1.14	1 / 1.15	-	-	-	-	-	-	-
Total		43 / 59.38	20 / 34.22	12 / 12.39	7 / 8.44	-	1 / 1.11	-	3 / 4.23	-

*#Indicates variants identified previously in cases of TKOS
†Allele counts are reported as Allele Count / Adjusted Allele Count

Table 3. Estimated Prevalence of Triadin Null Individuals

Ethnicity	Total Adjusted Allele Count	Total Individuals in gnomAD	Frequency of Individuals with a *TRDN* Null Variant	Frequency of Triadin Null Individuals
All	59.38	141,456	1:2382	~1:22.7 million
European (non-Finnish)	34.22	64,603	1:1888	~1:14.3 million
African	12.39	12,487	1:1008	~1:4.1 million
Latino	8.44	17,720	1:2100	~1:17.6 million
Ashkenazi Jewish	-	5,185	-	-
East Asian	1.11	9,977	1:8973	~1:322.1 million
European (Finnish)	-	12,526	-	-
South Asian	4.23	15,308	1:3621	~1:52.5 million
Other	-	3,614	-	-

Figure Legends:

Figure 1. Cardiac triadin topology with location of triadin null variants present in gnomAD Depicted is a schematic representation of *TRDN*-encoded cardiac triadin with all 16 of the triadin null variants found in gnomAD. Each white dot represents the location of a corresponding null variant. # indicates variants identified previously in cases of TKOS. The KEKE domain is the region known to be responsible for the interaction and binding of both calsequestrin and the ryanodine receptor (amino acids 210-224).