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GWAS Analysis of Sudden Cardiac Death Cases in a Turkish Population

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Abstract

Objective: Sudden death is defined as death occurring within one hour of the onset of symptoms, with cardiovascular diseases being one of the leading causes. The most common genetic factors leading to sudden cardiac death are hypertrophic cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy. In some cases, autopsies may reveal no evidence of long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia, or Brugada Syndrome.

Materials and Methods: We collected samples from sudden cardiac death cases aged 5–40 years (arrhythmia as Group 1, hypertrophy as Group 2, and ischemic heart disease as Group 3) , as well as from healthy athletes (control group as Group 4), and analyzed them using genome-wide association study (GWAS) with a DNA microchip containing 196,725 single nucleotide polymorphism (SNP) markers thought to be associated with sudden cardiac death or other cardiovascular diseases.

Results: We detected any possible genetic variations or patterns that could elucidate the mechanisms underlying sudden cardiac death in a Turkish population. In our study group, two polymorphisms; rs2971851 and rs9609516, stood out as prominent variants compared with healthy elite athletes.

Conclusion: We aimed to identify potential genetic variations or patterns that could shed light on the mechanisms underlying sudden cardiac death in the Turkish population. In our study group, two polymorphisms, rs2971851 and rs9609516, emerged as prominent variants when compared to healthy athletes.

Keywords Sudden cardiac death · Microarray analysis · GWAS · Genetics · Forensic medicine

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INTRODUCTION

In forensic medicine practices, sudden and unexpected deaths are the most frequent ones among natural death causes. It is reported that every year approximately 350,000 people in Europe and between 300,000 and 400,000 people in the USA die suddenly and unexpectedly (1). Moreover, it has been shown that most of these deaths are of cardiac origin (2). This phenomenon, known as sudden cardiac death, is defined as death occurring within one hour of the onset of cardiac symptoms—such as chest pain, palpitations, or fainting—in an otherwise healthy individual (3). While channelopathies and cardiomyopathies frequently cause sudden cardiac death in younger populations, coronary artery diseases are among the most common causes of sudden cardiac death in middle-aged individuals (4).

Sudden cardiac death is an important public health problem affecting the community and young people. Therefore, to prevent possible young deaths, it is important to understand the basic features and mechanisms of the disease. Consequently, genetic studies have gained importance in recent years. The genome-wide association study (GWAS) is a technique that has been used since 2007 to evaluate complex diseases and common diseases or population-based studies. The primary purpose of GWAS is to link potentially associated genes with a specific disease or trait. GWAS analysis is advantageous because it can include a large number of single nucleotide polymorphisms (SNPs) and can reliably analyze them in a short time with a large number of samples (5, 6).

For our study, we used a DNA chip containing 196,725 SNP markers associated with cardiovascular disease risk to identify polymorphic gene regions specific to the Turkish population and to uncover the relationships between genes and diseases.

MATERIALS AND METHODS

We selected 152 autopsies performed at the Forensic Medicine Institute between 2011 and 2013. These cases were associated with sudden cardiac death, were aged between 5 and 40 years, and had no previous cardiac complaints or family history of sudden cardiac death. Blood and tissue samples were stored at -20°C.

We divided selected cases into 3 groups according to clinical history and macroscopic and histopathological examination findings. Group 1 (n=53; 12 females and 41 males), "negative autopsy group (Group 1)" comprised cases with no structural changes in the heart and no occlusive lesions in their coronary arteries; Group 2 (n=50; 13 females and 37 males), "hypertrophic cardiomyopathy group" comprised of cases showing histopathological hypertrophic cardiomyopathy features in cardiac tissue; and Group 3 (n=49; 5 females and 44 males), "ischemic heart disease group" comprised cases showing occlusive features and histopathological ischemic features in heart tissue. Cases with a history of epilepsy, structural anomalies in the heart and/or coronary arteries (valvular malformation, coronary bridging, etc.), myocarditis, or toxicological features on chemical examination were excluded from the study. Group 4 was formed as the control group consisting of 80 healthy athletes.

The control group was selected using the following criteria to increase the scientific validity and statistical power of the results;

1) We selected individuals who did not have any heart disease in themselves or their family, have no history of sudden cardiac death in the family, have undergone echocardiogram (ECHO) and electrocardiogram (ECG) screenings, receive regular checkups, and engage in regular physical activity.
2) The samples were selected from individuals aged 16-40, as in the autopsy samples.
3) The gender distribution was adjusted to ensure a higher proportion of males in the control group, as sudden cardiac deaths are more commonly observed in males.
4) To avoid ethnic and geographical differences among individuals, the control group was selected from people living in Istanbul.

We performed total DNA isolation using an Invitrogen Mini Kit and a Qiagen Mini Blood Kit using the blood samples of the control group and tissue samples of the case groups collected during the autopsies. We measured the purity and concentration of the DNA samples by spectrophotometric method at a wavelength of 260/280 nm using a Nanodrop spectrophotometer. A minimum of 40 ng/µL concentration was needed for our purposes, and a total of 32 DNA isolates that did not reach this concentration were not included in further studies. 4-5 µL from each DNA isolate was used.

GWAS Analysis

We analyzed 196,725 SNP markers using an iScan Microarray Scanner (Illumina Inc®) and GenomeStudio®v2011.1 software (Illumina Inc®) for all preliminary analyses and quality analysis of the process.

We then compared the samples using the "Identity by Descent (IBD) Estimation" according to the alleles that they shared. The estimated proximity identification (PI) value indicates shared alleles. This value is expected to be one for monozygotic twins, 0.5 for siblings, and 0.25 for cousins. We verified the sex of the samples using the heterozygosity of the X chromosome, which was determined during the genotyping process.

The processed data were transferred from GenomeStudio to Golden Helix®SNP & Variation Suite (SVS). We started the analyses with 196,725 SNP markers, but after removing the markers on chromosome Y (due to the fact that genes on the Y chromosome are associated with very few diseases), we continued with 185,802 SNP markers.

Statistical Analyses

We applied Fisher's exact test for Hardy-Weinberg Equilibrium (HWE), and markers with p values lower than 10^{-5} and SNP markers with Linkage Disequilibrium (LD) (>0.5) were filtered out and excluded from the analysis.

We started whole genome association analysis using the markers determined during the SNP filtering process. "Additive model, (dd)>(Dd)>(DD)" and applied "Correlation/ Trend" test were selected. We used Bonferroni Adjustment and False Discovery Rate (FDR) tests as Multiple Correction tests.

We performed the statistical analyses using the SPSS 20.0 package program (IBM Corp., Armonk, NY, USA) and used p<0.05 as significance limit.

RESULTS

Group 1 consisted of 53 (12 females and 41 males, mean age=23.13), Group 2 included 50 (13 females and 37 males, mean age=28.94), and Group 3 included 49 (5 females and 44 males, mean age=35.22) cases.

Information about the activities of the cases at the time of death are given in Table 1.

Table 1. Distribution of patient groups by place of death

Activity at the Time of Death	Frequency	Percentage	Valid Percentage	Cumulative Percentage
Unknown	9	5.9	5.9	5.9
Resting	50	32.9	32.9	38.8
Driving	4	2.6	2.6	41.4
Sleeping	8	5.3	5.3	46.7
Found dead at home	22	14.5	14.5	61.2
Found dead in the bathroom	9	5.9	5.9	67.1
At workplace	9	5.9	5.9	73
During argument	2	1.3	1.3	74.3
During physical effort	34	22.4	22.4	96.7
Few days lasting chest pain	5	3.3	3.3	100
Total	152	100	100	

Evaluation of the Microarray Findings

Group 1 (arrhythmia), Group 2 (hypertrophy), and Group 3 (ischemic heart disease) were included in the analysis. Samples in Group 3 were analyzed separately as hypertrophy-coronary heart disease and hypertrophy-coronary heart disease-myocardial infarction, yet evaluated as a single group. We examined a total of 200 DNA samples, including case groups; however, in 60 samples data acquisition (call rates) were detected below 95%, and therefore these were excluded from the study. Among the 140 samples, the highest data acquisition was determined as 0.9969, and the lowest as 0.9557.

Proximity Identification (PI)

We found the estimated PI value of these 140 control samples as 0.097, and since there were no kinship between the samples, none were excluded from the analysis.

Gender Determination

We determined the sex of the samples using heterozygosity data from the X chromosome. The gender distribution of the genotyping samples was 66 male and 16 female cases. In the control group, 44 were males and 14 were females.

SNP Filtering

We began our analysis using 196,725 SNP markers across a total of 140 samples. Subsequently, we removed the markers on the Y-chromosome and continued with the remaining 185,802 markers. The number of markers with a genotyping rate below 95% was 10,596. The number of markers with minor allele frequency (MAF) below 0.01 was 60,232. Markers with a p value of less than 10^{-5} for HWE were 507, and therefore, a total of 68,688 SNP markers were excluded from the analysis (7).

Linkage Disequilibrium (LD)

A total of 57,569 SNP markers with LD (>0.5) were excluded from the analysis.

GWAS Analysis

Following SNP filtering, we analyzed 140 samples (82 cases, 58 controls) with 59,545 SNP markers. As a result of the analysis, among the SNPs with a p value below $1x10^{-4}$, the ones with the highest significance were determined as rs2971851 on the 2. chromosome (2p14, MAF=0.7342) and rs9609516 on the 22. chromosome (22q12.3, MAF=0.1273).

Evaluation of Results According to Case-Control Relationship

The Q-Q chart of the expected and observed values is shown in Figure 1. The Manhattan Plot for case groups and control group is shown in Figure 2. SNP markers with a p value below

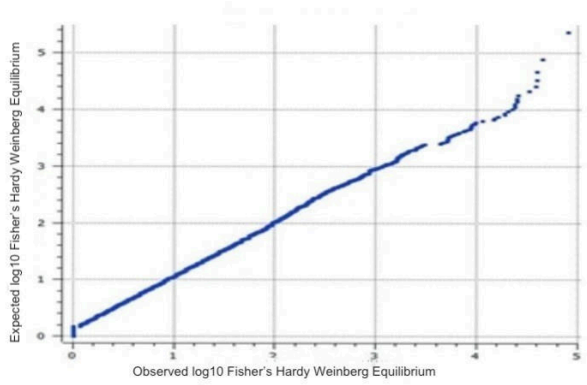

Figure 1. The Q-Q plot of the expected and observed values among the case and control groups

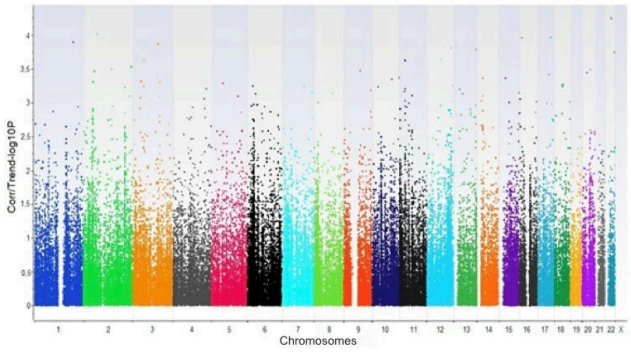

Figure 2. Manhattan Plot demonstration between case/control groups

1x10^{-4} according to the case groups-control relationship are presented in Tables 2, 3, and 4.

Table 2. SNPs associated with arrhythmia (Group 1) compared to controls (Group 4)

Marker	Chromosome	Gene	Position	Allele Change	The Minor Allele Frequency	Variant
rs2369527	1q31.2	*LINC02770*	191872334	A/G	0.1663	Intronic
rs2632594	3p22,1	*ULK4*	41480682	A/G	0.8922	Intronic
rs10213562	4q25	*MCUB*	110494732	T/G	0.2146	Intronic
rs37569	5q11.2	*PDE4D*	58839567	A/C	0.8432	Intronic
rs12209155	6p12.3	*PTCHD4*	47891704	C/T	0.1204	Intronic
rs10811461	9p21,3	*NONE*	21063183	G/A	0.1246	NONE
rs3786189	18q23	*NFATC1*	77201837	T/C	0.2533	Intronic

Table 3. SNPs associated with hypertrophic cardiomyopathy (Group 2) compared controls (Group 4)

Marker	Chromosome	Gene	Position	Allele Change	The Minor Allele Frequency	Variant
rs792232	10q23.31	*RNLS*	90147344	G/A	0.8886	Intronic
rs1172479	10q24.1	*PIK3AP1*	98481307	T/A	0.3851	Upstream
rs9609516	22q12.3	*RFPL3*	32755074	G/T	0.1273	Intronic

Table 4. SNPs associated with ischemic coronary artery disease (Group 3) compared controls (Group 4)

Marker	Chromosome	Gene	Position	Allele Change	The Minor Allele Frequency	Variant
rs7593239	2q34	LOC101927960	209605944	G/A	0.1387	Intronic
rs3856953	4p16.2	*EVC/ CRMP1*	5774747	C/A	0.9373	Intronic
rs6822202	4q32.3	*MARCHF1*	165132898	G/A	0.08282	Intronic
rs17440042	4p14	*N4BP2*	40130593	G/A	0.07490	Intronic
rs488174	5q13.2	LOC105379030	72429346	C/T	0.1095	Intronic
rs6864267	5q32	*PDE6A*	149247738	C/A	0.05700	Intronic
rs1428507	5q34	*NONE*	164791450	G/A	0.1918	NONE
rs994690	6p22,2	*NONE*	27047916	T/C	0.08668	NONE
rs6456769	6p22,1	*H2BC12*	27107865	G/A	0.08692	Intronic
rs10499295	6q25.3	LOC101928923	156223473	G/T	0.05196	Intronic
rs1107152	8q13.2	*PREX2*	68896865	A/G	0.4214	Intronic
rs10088446	8q13.2	*PREX2*	68898599	G/A	0.3783	Intronic
rs12115844	9q34.11	*FNBP1*	132720869	C/T	0.04864	Intronic
rs9424135	10p15,1	*ASB13*	5700843	G/T	0.09645	Intronic
rs2094248	13q13.3	*DCLK1*	36483466	A/G	0.09936	Intronic
rs9919897	14q11.2	LOC105370401	22862876	T/C	0.7667	Intronic
rs1034377	14q11.2	LOC105370401	22865841	A/G	0.8001	Intronic
rs2014778	14q11.2	LOC105370401	22876816	G/T	0.7081	Intronic
rs760017	14q11.2	LOC105370401	22882590	A/C	0.7063	Intronic
rs226785	16q13.12	*MRTFB*	14313728	G/A	0.05145	Intronic
rs4782921	16q24.1	*WFDC1*	84360361	C/A	0.1796	Intronic
rs1641788	16p13.13	*NUBP1/ TVP23A*	10861110	C/T	0.1796	Intronic /3'UTR
rs1468753	17q22	*AKAP1*	55162325	T/G	0.9023	Upstream
rs8111989	19q13.32	*CKM*	45809208	T/C	0.3532	Downstream
rs9619601	22q12.3	*MYH9*	36700175	A/G	0.05311	Synonym

In our study, we selected 13 polymorphisms that have been shown to be associated with the cardiac diseases and that were observed in at least one and at most 3 patients in the same group. These polymorphisms are shown in Table 5.

DISCUSSION

In our study on the prevalence of sudden cardiac deaths in the population, the results were consistent with the literature, indicating that the death rate among male is higher than that among female. This difference is considered to be due to the protective effects of the estrogen hormone on blood vessels (8).

In our study, the location of death in autopsy cases was significant in terms of its relevance to the existing literature. In this classification, since fatal symptoms were reported to occur in the bed, bathroom, and toilet, the classification was

Table 5. Candidate variations for cases (Group 1-3)

Marker	Chromosome	Gene	Coordinate	Allele Change	The Minor Allele Frequency	Changes in Amino Acid Levels	Pathogenicity	Variant
rs78121716	11p11.2	*NDUFS3*	47605891	G/A	0.00003976	p.Arg218Gln	VUS	Missense
rs61742331	2p24.1	*APOB*	21229679	G/C	0.0008392	p.Ala3354Gly	CIP	Missense
rs72653074	2p24.1	*APOB*	21239423	C/T	0.00002121	p.Gly1074Arg	VUS	Missense
rs61742990	2p24.1	*APOB*	21255262	C/T	0.00003541	p.Arg439Gln	VUS	Missense
rs72653102	2p24.1	*APOB*	21230334	G/A	0.00004782	p.Arg3136Cys	VUS	Missense
rs59827137	1q24.2	*NME7*	169138708	G/T	0.002091	p.Leu359Met		Missense
rs76757832	6p22.2	*TRIM38*	25969631	C/G	0.000004042	p.Arg164Gly		Missense
rs183414771	3q27.2	*IGF2BP2*	185393659	C/G	0.0001592	p.Leu285Phe		Missense
rs141107387	3p22.2	*SCN5A*	38592107	C/T	0.000008024	p.Arg1918His	VUS	Missense
rs35310697	9p21.3	*DMRTA1*	22451581	G/T	0.001811	p.Ala396Thr		Missense
rs3782886	12q24.12	*BRAP*	112110489	T/C	0.01867	p.Glu4Gly		Missense
rs11575933	9q34.2	*ADAMTS13*	136302063	C/T	0.005366	p.Pro475Ser	CIP	Missense
rs1800562	6p22.2	*HFE*	26093141	G/A	0.03377	p.Cys282Tyr	Pathogenic	Missense

VUS: Variant of Uncertain Significance; CIP: Conflicting Interpretations of Pathogenicity

made as death at home, death in the bed, and death in the bathroom (9).

Since the autopsy materials in our study were selected from cases sent to the Istanbul Morgue Department of the Council of Forensic Medicine, it was not possible to clearly reflect the regional distribution of sudden cardiac deaths. Therefore, a regional evaluation was not conducted.

In addition to many family studies, population-based studies have been carried out in relation to sudden cardiac deaths. Studies on the genetic background of diseases such as cardiovascular disease and cancer have been conducted with GWAS since 2007. The advantage of GWAS is that it allows a large number of samples to be run quickly on microchips with a large number of SNPs (6).

In our study, we found the SNPs rs2971851 and rs2971851 to be associated with the sudden cardiac death; however, no association with the cardiac diseases has been reported in either polymorphism.

When the cases were grouped according to their histopathological findings, the polymorphisms rs2369527, rs2632594, rs10213562, rs37569, rs12209155, rs10811461, and rs3786189 in the arrhythmia group were significantly associated with sudden cardiac death compared with the control group. None of these polymorphisms were previously associated with cardiac patients. However, mutations in the *NFATc1* gene with rs3786189 were associated with congenital cardiac diseases (10); mutations in the *ULK4* gene with rs2632594 were associated with hypertension (11) and acute aortic dissection (12); mutations in the *MCUB* gene with rs10213562 (12, 13) with ischemic heart disease; and mutations in the *PDE4D* gene with rs37569 were associated with arrhythmogenic cardiac diseases (14, 15).

A significant correlation was found between rs792232, rs1172479, and rs9609516 and sudden cardiac death in the hypertrophic cardiomyopathy group. Previously, none of these polymorphisms were associated with the cardiac events. However, mutations in the *RNLS* gene with rs792232 polymorphism have been associated with hypertension (16) and type 1 Diabetes Mellitus (17).

A significant correlation was observed with sudden cardiac death and rs7593239, rs3856953, rs6822202, rs17440042, rs488174, rs6864267, rs1428507, rs994690, rs6456769, rs6456769, rs10499295, rs1107152, rs10088446, rs12115844, rs9424135, rs2094248, rs9919897, rs1034377, rs2014778, rs760017, rs226785, rs478292, rs1641788, rs1468753, and rs8111989 in the coronary artery group. However, none of these polymorphisms had been reported in the literature to be associated with the cardiac diseases. Among these, rs6864267 has been stated as a benign variant in the ClinVar database. On the other hand, there are no mutations associated with cardiovascular diseases reported in the *PDE6A* gene, in which it is found. The *MRTFB* gene, in which the rs226785 polymorphism is found, is called Myocardin Related Transcription Factor B and is known to play an active role in heart development (18).

It has been reported that the *AKAP1* gene, in which rs1468753 is found, plays a role in the development of cardiac hypertrophy, hypoxia-induced myocardial infarction, and endothelial cell dysfunction (19, 20).

In our study, we selected 13 polymorphisms that have been associated with the cardiac diseases and observed in at least

one and at most 3 patients in the same group. Among these variants, it has been reported that the rs1801278 (p.Gly971Arg) variant in the *IRS1* gene may be a risk factor for coronary artery disease, especially with the history of diabetes (21, 22).

Although there is no literature on rs72653074 (p.Gly1074Arg), 724742990 (p.Arg439Gln), and rs72653102 (p.Arg3136Cys) in the *APOB* gene, all of them were detected among different patients in Group 3, and their clinical importance in the ClinVar database is stated as conflicting classifications of pathogenicity (CIP) for familial hypercholesterolemia patients (23). Although there is no literature on rs141107387 (p.Arg1918His) in the *SCN5A* gene, which was detected in 3 different patients in Group 1, they were reported as CIP for clinically important hereditary arrhythmic diseases in the ClinVar database (23).

The rs61742331 (p.Ala3354Gly) variant of the *APOB* gene, which was found in only one patient in Group 3, has been reported in the ClinVar database as CIP for clinically important familial hypercholesterolemia disease (23, 24). The rs11575933 (p.Pro475Ser) variant of the *ADAMTS13* gene, found in only one patient in Group 3, has been proposed as a pathogenic variant for hereditary thrombotic thrombocytopenic purpura by Kokame et al. and Akiyma et al.(25, 26).

The rs1800562 (p.Cys282Tyr) variant of the *HFE* gene, found in only one patient in Group 2, has been suggested to be a pathogenic variant of cardiomyopathy (23) and Familial Hemochromatosis Syndrome (27-31). In addition, it has been reported that the same variant is a risk factor for microvascular complications in Type II Diabetes patients (32, 33). Furthermore, the same variant was suggested as a possible pathogenic variant for Cardiomyopathy in the Clinvar database (23).

In addition to all these, variants of rs74522665 (p.Gly1386Arg) of *NOTCH2* found in only 1 case in Group 1, that is known to play a role in cardiac development, rs59827137 (p.Leu359Met) of *NME7* found in 1 case in Group 2, rs76757832 (p.Arg164Gly) on *TRIM38* found in 1 case in Group 1, rs183414771 (p.Leu285Phe) of *IGF2BP2* found in 1 case in Group 1, rs8176721 (p.Asn105Lys) of *APOB* found in 1 case in Group 1, rs35310697 (p.Ala396Thr) of *DMRTA1* found in one case each of Group 1, 2 and 3, rs115553210 (p.Trp195Ter) of *REXO4* found in one case in Group 1, rs3782886 (p.Glu4Gly) of *BRAP* found in one case in Group 1, rs114104180 (p.Glu1218Asp) of *HECTD4* found in one case each in Group 1 and 2, rs117505183 (p.Arg2511Trp) of *HECTD4* found in one case in Group 1, rs188294925 (p.Arg528Gly) of *GNL3* found in one case in Group 1 should be further evaluated in terms of sudden cardiac death.

CONCLUSION

The development of new strategies for the early diagnosis and prevention of sudden cardiac death is important for public health. Understanding the molecular mechanisms underlying sudden cardiac death represents a crucial step in this process. The results of the GWAS of our study suggest new and possibly complementary biological pathways that may be involved in sudden cardiac death. Furthermore, our study is the first autopsy-based research to investigate variants observed in the Turkish population in cases of sudden cardiac death. In future studies, sequencing analyses of these candidate genes should be performed to look for possible mutations. By determining the sudden cardiac death gene profile specific to Turkish population, it will be possible to determine the people who inherit the disease and the people who develop it spontaneously; and to take the necessary precautions in terms of the risk of sudden cardiac death.

Ethics Committee Approval	This study was approved by the Local Ethics Committee of Istanbul Faculty of Medicine (28.07.2011-805).
Peer Review	Externally peer-reviewed.
Conception/Design of Study	G.O.C., K.K.; Data Acquisition: G.O.C., M.C., K.K., A.B.C., A.P.E., I.K., B.S., M.Y., B.B., H.Y.A., O.O.; Data Analysis/Interpretation: G.O.C., M.C., K.K., A.B.C., A.P.E., I.K., B.S., M.Y., B.B., H.Y.A., O.O.; Drafting Manuscript: G.O.C., K.K., A.P.E.; Critical Revision of Manuscript: G.O.C., M.C., K.K., A.B.C., A.P.E., I.K., B.S., M.Y., B.B., H.Y.A., O.O.; Final Approval and Accountability: G.O.C., M.C., K.K., A.B.C., A.P.E., I.K., B.S., M.Y., B.B., H.Y.A., O.O.
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