References

1. Lopshire JC, Zipes DP. Sudden cardiac death: better understanding of risks, mechanisms, and treatment. *Circulation*. 2006;114:1134–1136. doi: 10.1161/CIRCULATIONAHA.106.647933.
2. Fishman GI, Chugh SS, Dimarco JP, et al. Sudden cardiac death prediction and prevention: report from a National Heart, Lung, and Blood Institute and Heart Rhythm Society Workshop. *Circulation*. 2010;122:2335–2348. doi: 10.1161/CIRCULATIONAHA.110.976092.
3. Hinkle LE Jr, Thaler HT. Clinical classification of cardiac deaths. *Circulation*. 1982;65:457–464.
4. Weisfeldt ML, Everson-Stewart S, Sitlani C, Rea T, Aufderheide TP, Atkins DL, Bigham B, Brooks SC, Foerster C, Gray R, Ornato JP, Powell J, Kudenchuk PJ, Morrison LJ; Resuscitation Outcomes Consortium Investigators. Ventricular tachyarrhythmias after cardiac arrest in public versus at home. *N Engl J Med*. 2011;364:313–321. doi: 10.1056/NEJMoa1010663.
5. Albert CM, Chae CU, Grodstein F, Rose LM, Rexrode KM, Ruskin JN, Stampfer MJ, Manson JE. Prospective study of sudden cardiac death among women in the United States. *Circulation*. 2003;107:2096–2101. doi: 10.1161/01.CIR.0000065223.21530.11.
6. Gillum RF. Geographic variation in sudden coronary death. *Am Heart J*. 1990;119:380–389.
7. Myerburg RJ, Castellanos A. Sudden cardiac death. In: Zipes DP, Jalife J, eds. *Cardiac electrophysiology: From cell to bedside. 5th edn*. Philadelphia, PA: Saunders Elsevier; 2009:797–808.
8. Niemeijer MN, van den Berg ME, Leening MJG, Hofman A, Franco OH, Deckers JW, Heeringa J, Rijnbeek PR, Stricker BH, Eijgelsheim M. Declining incidence of sudden cardiac death from 1990–2010 in a general middle-aged and elderly population: The rotterdam study. *Heart Rhythm*. 2015;12:123–129.
9. Fox CS, Evans JC, Larson MG, Kannel WB, Levy D. Temporal trends in coronary heart disease mortality and sudden cardiac death from 1950 to 1999: the Framingham Heart Study. *Circulation*. 2004;110:522–527. doi: 10.1161/01.CIR.0000136993.34344.41.
10. Zheng ZJ, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. *Circulation*. 2001;104:2158–2163.
11. Dudas K, Lappas G, Stewart S, Rosengren A. Trends in out-of-hospital deaths due to coronary heart disease in Sweden (1991 to 2006). *Circulation*. 2011;123:46–52. doi: 10.1161/CIRCULATIONAHA.110.964999.
12. Gerber Y, Jacobsen SJ, Frye RL, Weston SA, Killian JM, Roger VL. Secular trends in deaths from cardiovascular diseases: a 25-year community study. *Circulation*. 2006;113:2285–2292. doi: 10.1161/CIRCULATIONAHA.105.590463.
13. Cupples LA, Gagnon DR, Kannel WB. Long- and short-term risk of sudden coronary death. *Circulation*. 1992;85:I11–I18.
14. Gorgels AP, Gijsbers C, de Vreede-Swagemakers J, Lousberg A, Wellens HJ. Out-of-hospital cardiac arrest–the relevance of heart failure. The Maastricht Circulatory Arrest Registry. *Eur Heart J*. 2003;24:1204–1209.
15. Kong MH, Fonarow GC, Peterson ED, Curtis AB, Hernandez AF, Sanders GD, Thomas KL, Hayes DL, Al-Khatib SM. Systematic review of the incidence of sudden cardiac death in the United States. *J Am Coll Cardiol*. 2011;57:794–801. doi: 10.1016/j.jacc.2010.09.064.
16. Myerburg RJ, Junttila MJ. Sudden cardiac death caused by coronary heart disease. *Circulation*. 2012;125:1043–1052. doi: 10.1161/CIRCULATIONAHA.111.023846.
17. Berdowski J, Berg RA, Tijssen JG, Koster RW. Global incidences of out-of-hospital cardiac arrest and survival rates: Systematic review of 67 prospective studies. *Resuscitation*. 2010;81:1479–1487. doi: 10.1016/j.resuscitation.2010.08.006.
18. Chugh SS, Jui J, Gunson K, Stecker EC, John BT, Thompson B, Ilias N, Vickers C, Dogra V, Daya M, Kron J, Zheng ZJ, Mensah G, McAnulty J. Current burden of sudden cardiac death: multiple source surveillance versus retrospective death certificate-based review in a large U.S. community. *J Am Coll Cardiol*. 2004;44:1268–1275. doi: 10.1016/j.jacc.2004.06.029.
19. Zipes DP, Camm AJ, Borggrefe M, et al; American College of Cardiology/American Heart Association Task Force; European Society of Cardiology Committee for Practice Guidelines; European Heart Rhythm Association; Heart Rhythm Society. ACC/AHA/ESC 2006 Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (writing committee to develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. *Circulation*. 2006;114:e385–e484. doi: 10.1161/CIRCULATIONAHA.106.178233.
20. de Vreede-Swagemakers JJ, Gorgels AP, Dubois-Arbouw WI, van Ree JW, Daemen MJ, Houben LG, Wellens HJ. Out-of-hospital cardiac arrest in the 1990's: a population-based study in the Maastricht area on incidence, characteristics and survival. *J Am Coll Cardiol*. 1997;30:1500–1505.
21. Byrne R, Constant O, Smyth Y, Callagy G, Nash P, Daly K, Crowley J. Multiple source surveillance incidence and aetiology of out-of-hospital sudden cardiac death in a rural population in the West of Ireland. *Eur Heart J*. 2008;29:1418–1423. doi: 10.1093/eurheartj/ehn155.
22. Hua W, Zhang LF, Wu YF, Liu XQ, Guo DS, Zhou HL, Gou ZP, Zhao LC, Niu HX, Chen KP, Mai JZ, Chu LN, Zhang S. Incidence of sudden cardiac death in China: analysis of 4 regional populations. *J Am Coll Cardiol*. 2009;54:1110–1118. doi: 10.1016/j.jacc.2009.06.016.
23. Winkel BG, Holst AG, Theilade J, Kristensen IB, Thomsen JL, Ottesen GL, Bundgaard H, Svendsen JH, Haunsø S, Tfelt-Hansen J. Nationwide study of sudden cardiac death in persons aged 1-35 years. *Eur Heart J*. 2011;32:983–990. doi: 10.1093/eurheartj/ehq428.
24. Margey R, Roy A, Tobin S, O'Keane CJ, McGorrian C, Morris V, Jennings S, Galvin J. Sudden cardiac death in 14- to 35-year olds in Ireland from 2005 to 2007: a retrospective registry. *Europace*. 2011;13:1411–1418. doi: 10.1093/europace/eur161.
25. Papadakis M, Sharma S, Cox S, Sheppard MN, Panoulas VF, Behr ER. The magnitude of sudden cardiac death in the young: a death certificate-based review in England and Wales. *Europace*. 2009;11:1353–1358. doi: 10.1093/europace/eup229.
26. Shojania KG, Burton EC. The vanishing nonforensic autopsy. *N Engl J Med*. 2008;358:873–875. doi: 10.1056/NEJMp0707996.
27. Lunetta P, Lounamaa A, Sihvonen S. Surveillance of injury-related deaths: medicolegal autopsy rates and trends in Finland. *Inj Prev*. 2007;13:282–284. doi: 10.1136/ip.2006.012922.
28. Field JM, Hazinski MF, Sayre MR, et al. Part 1: executive summary: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation*. 2010;122:S640–S656. doi: 10.1161/CIRCULATIONAHA.110.970889.
29. Chan PS, McNally B, Tang F, Kellermann A; CARES Surveillance Group. Recent trends in survival from out-of-hospital cardiac arrest in the United States. *Circulation*. 2014;130:1876–1882. doi: 10.1161/CIRCULATIONAHA.114.009711.
30. Wissenberg M, Lippert FK, Folke F, et al. Association of national initiatives to improve cardiac arrest management with rates of bystander intervention and patient survival after out-of-hospital cardiac arrest. *JAMA*. 2013;310:1377–1384. doi: 10.1001/jama.2013.278483.
31. Cobb LA, Fahrenbruch CE, Olsufka M, Copass MK. Changing incidence of out-of-hospital ventricular fibrillation, 1980-2000. *JAMA*. 2002;288:3008–3013.
32. Nichol G, Thomas E, Callaway CW, Hedges J, Powell JL, Aufderheide TP, Rea T, Lowe R, Brown T, Dreyer J, Davis D, Idris A, Stiell I; Resuscitation Outcomes Consortium Investigators. Regional variation in out-of-hospital cardiac arrest incidence and outcome. *JAMA*. 2008;300:1423–1431. doi: 10.1001/jama.300.12.1423.
33. Myerburg RJ, Halperin H, Egan DA, et al. Pulseless electric activity: definition, causes, mechanisms, management, and research priorities for the next decade: report from a National Heart, Lung, and Blood Institute workshop. *Circulation*. 2013;128:2532–2541. doi: 10.1161/CIRCULATIONAHA.113.004490.
34. Straus SM, Bleumink GS, Dieleman JP, van der Lei J, Stricker BH, Sturkenboom MC. The incidence of sudden cardiac death in the general population. *J Clin Epidemiol*. 2004;57:98–102. doi: 10.1016/S0895-4356(03)00210-5.
35. Kuisma M, Repo J, Alaspää A. The incidence of out-of-hospital ventricular fibrillation in Helsinki, Finland, from 1994 to 1999. *Lancet*. 2001;358:473–474. doi: 10.1016/S0140-6736(01)05634-3.
36. Krahn AD, Connolly SJ, Roberts RS, Gent M; ATMA Investigators. Diminishing proportional risk of sudden death with advancing age: implications for prevention of sudden death. *Am Heart J*. 2004;147:837–840. doi: 10.1016/j.ahj.2003.12.017.
37. Iwami T, Hiraide A, Nakanishi N, Hayashi Y, Nishiuchi T, Yukioka H, Yoshiya I, Sugimoto H. Age and sex analyses of out-of-hospital cardiac arrest in Osaka, Japan. *Resuscitation*. 2003;57:145–152.
38. Kannel WB, Wilson PW, D'Agostino RB, Cobb J. Sudden coronary death in women. *Am Heart J*. 1998;136:205–212. doi: 10.1053/hj.1998.v136.90226.
39. Rho RW, Patton KK, Poole JE, Cleland JG, Shadman R, Anand I, Maggioni AP, Carson PE, Swedberg K, Levy WC. Important differences in mode of

death between men and women with heart failure who would qualify for a primary prevention implantable cardioverter-defibrillator. *Circulation*. 2012;126:2402–2407. doi: 10.1161/CIRCULATIONAHA.111.069245.
40. Wissenberg M, Hansen CM, Folke F, Lippert FK, Weeke P, Karlsson L, Rajan S, Søndergaard KB, Kragholm K, Christensen EF, Nielsen SL, Køber L, Gislason GH, Torp-Pedersen C. Survival after out-of-hospital cardiac arrest in relation to sex: a nationwide registry-based study. *Resuscitation*. 2014;85:1212–1218. doi: 10.1016/j.resuscitation.2014.06.008.
41. Akahane M, Ogawa T, Koike S, Tanabe S, Horiguchi H, Mizoguchi T, Yasunaga H, Imamura T. The effects of sex on out-of-hospital cardiac arrest outcomes. *Am J Med*. 2011;124:325–333. doi: 10.1016/j.amjmed.2010.10.020.
42. Becker LB, Han BH, Meyer PM, Wright FA, Rhodes KV, Smith DW, Barrett J. Racial differences in the incidence of cardiac arrest and subsequent survival. The CPR Chicago Project. *N Engl J Med*. 1993;329:600–606. doi: 10.1056/NEJM199308263290902.
43. Cowie MR, Fahrenbruch CE, Cobb LA, Hallstrom AP. Out-of-hospital cardiac arrest: racial differences in outcome in Seattle. *Am J Public Health*. 1993;83:955–959.
44. Bertoia ML, Allison MA, Manson JE, Freiberg MS, Kuller LH, Solomon AJ, Limacher MC, Johnson KC, Curb JD, Wassertheil-Smoller S, Eaton CB. Risk factors for sudden cardiac death in post-menopausal women. *J Am Coll Cardiol*. 2012;60:2674–2682. doi: 10.1016/j.jacc.2012.09.031.
45. Okin PM, Kjeldsen SE, Julius S, Dahlöf B, Devereux RB. Racial differences in sudden cardiac death among hypertensive patients during antihypertensive therapy: the LIFE study. *Heart Rhythm*. 2012;9:531–537. doi: 10.1016/j.hrthm.2011.11.008.
46. Shah KS, Shah AS, Bhopal R. Systematic review and meta-analysis of out-of-hospital cardiac arrest and race or ethnicity: black US populations fare worse. *Eur J Prev Cardiol*. 2014;21:619–638. doi: 10.1177/2047487312451815.
47. Teodorescu C, Reinier K, Dervan C, Uy-Evanado A, Samara M, Mariani R, Gunson K, Jui J, Chugh SS. Factors associated with pulseless electric activity versus ventricular fibrillation: the Oregon sudden unexpected death study. *Circulation*. 2010;122:2116–2122. doi: 10.1161/CIRCULATIONAHA.110.966333.
48. Chan PS, Nichol G, Krumholz HM, Spertus JA, Jones PG, Peterson ED, Rathore SS, Nallamothu BK; American Heart Association National Registry of Cardiopulmonary Resuscitation (NRCPR) Investigators. Racial differences in survival after in-hospital cardiac arrest. *JAMA*. 2009;302:1195–1201. doi: 10.1001/jama.2009.1340.
49. Sasson C, Magid DJ, Chan P, Root ED, McNally BF, Kellermann AL, Haukoos JS; CARES Surveillance Group. Association of neighborhood characteristics with bystander-initiated CPR. *N Engl J Med*. 2012;367:1607–1615. doi: 10.1056/NEJMoa1110700.
50. Willey JZ, Rodriguez CJ, Moon YP, Paik MC, Di Tullio MR, Homma S, Sacco RL, Elkind MS. Coronary death and myocardial infarction among Hispanics in the Northern Manhattan Study: exploring the Hispanic paradox. *Ann Epidemiol*. 2012;22:303–309. doi: 10.1016/j.annepidem.2012.02.014.
51. Gillum RF. Sudden cardiac death in Hispanic Americans and African Americans. *Am J Public Health*. 1997;87:1461–1466.
52. Steinhaus DA, Vittinghoff E, Moffatt E, Hart AP, Ursell P, Tseng ZH. Characteristics of sudden arrhythmic death in a diverse, urban community. *Am Heart J*. 2012;163:125–131. doi: 10.1016/j.ahj.2011.09.016.
53. Maruyama M, Ohira T, Imano H, Kitamura A, Kiyama M, Okada T, Maeda K, Yamagishi K, Noda H, Ishikawa Y, Shimamoto T, Iso H. Trends in sudden cardiac death and its risk factors in Japan from 1981 to 2005: The circulatory risk in communities study (circs). *BMJ open*. 2012;2:e000573.
54. SPAIN DM, BRADESS VA, MOHR C. Coronary atherosclerosis as a cause of unexpected and unexplained death. An autopsy study from 1949-1959. *JAMA*. 1960;174:384–388.
55. Albert CM, McGovern BA, Newell JB, Ruskin JN. Sex differences in cardiac arrest survivors. *Circulation*. 1996;93:1170–1176.
56. Burke AP, Farb A, Pestaner J, Malcom GT, Zieske A, Kutys R, Smialek J, Virmani R. Traditional risk factors and the incidence of sudden coronary death with and without coronary thrombosis in blacks. *Circulation*. 2002;105:419–424.
57. Nagata M, Ninomiya T, Doi Y, Hata J, Ikeda F, Mukai N, Tsuruya K, Oda Y, Kitazono T, Kiyohara Y. Temporal trends in sudden unexpected death in a general population: the Hisayama study. *Am Heart J*. 2013;165:932–938.e1. doi: 10.1016/j.ahj.2013.02.028.
58. Priori SG, Borggrefe M, Camm AJ, Hauer RN, Klein H, Kuck KH, Schwartz PJ, Touboul P, Wellens HJ. Unexplained cardiac arrest. The need for a prospective registry. *Eur Heart J*. 1992;13:1445–1446.
59. Chugh SS, Chung K, Zheng ZJ, John B, Titus JL. Cardiac pathologic findings reveal a high rate of sudden cardiac death of undetermined etiology in younger women. *Am Heart J*. 2003;146:635–639. doi: 10.1016/S0002-8703(03)00323-5.
60. Murakoshi N, Aonuma K. Epidemiology of arrhythmias and sudden cardiac death in Asia. *Circ J*. 2013;77:2419–2431.
61. Kaltman JR, Thompson PD, Lantos J, et al. Screening for sudden cardiac death in the young: report from a national heart, lung, and blood institute working group. *Circulation*. 2011;123:1911–1918. doi: 10.1161/CIRCULATIONAHA.110.017228.
62. Greene HL. Sudden arrhythmic cardiac death–mechanisms, resuscitation and classification: the Seattle perspective. *Am J Cardiol*. 1990;65:4B–12B.
63. Eisenberg MS, Copass MK, Hallstrom AP, Blake B, Bergner L, Short FA, Cobb LA. Treatment of out-of-hospital cardiac arrests with rapid defibrillation by emergency medical technicians. *N Engl J Med*. 1980;302:1379–1383. doi: 10.1056/NEJM198006193022502.
64. Jouven X, Desnos M, Guerot C, Ducimetière P. Predicting sudden death in the population: the Paris Prospective Study I. *Circulation*. 1999;99:1978–1983.
65. Wannamethee G, Shaper AG, Macfarlane PW, Walker M. Risk factors for sudden cardiac death in middle-aged British men. *Circulation*. 1995;91:1749–1756.
66. Kucharska-Newton AM, Couper DJ, Pankow JS, Prineas RJ, Rea TD, Sotoodehnia N, Chakravarti A, Folsom AR, Siscovick DS, Rosamond WD. Diabetes and the risk of sudden cardiac death, the Atherosclerosis Risk in Communities study. *Acta Diabetol*. 2010;47(Suppl 1):161–168. doi: 10.1007/s00592-009-0157-9.
67. Junttila MJ, Barthel P, Myerburg RJ, Mäkikallio TH, Bauer A, Ulm K, Kiviniemi A, Tulppo M, Perkiömäki JS, Schmidt G, Huikuri HV. Sudden cardiac death after myocardial infarction in patients with type 2 diabetes. *Heart Rhythm*. 2010;7:1396–1403. doi: 10.1016/j.hrthm.2010.07.031.
68. Deo R, Vittinghoff E, Lin F, Tseng ZH, Hulley SB, Shlipak MG. Risk factor and prediction modeling for sudden cardiac death in women with coronary artery disease. *Arch Intern Med*. 2011;171:1703–1709. doi: 10.1001/archinternmed.2011.328.
69. Mayet J, Shahi M, Foale RA, Poulter NR, Sever PS, McG Thom SA. Racial differences in cardiac structure and function in essential hypertension. *BMJ*. 1994;308:1011–1014.
70. Vlietstra RE, Kronmal RA, Oberman A, Frye RL, Killip T 3rd. Effect of cigarette smoking on survival of patients with angiographically documented coronary artery disease. Report from the CASS registry. *JAMA*. 1986;255:1023–1027.
71. Goldenberg I, Jonas M, Tenenbaum A, Boyko V, Matetzky S, Shotan A, Behar S, Reicher-Reiss H; Bezafibrate Infarction Prevention Study Group. Current smoking, smoking cessation, and the risk of sudden cardiac death in patients with coronary artery disease. *Arch Intern Med*. 2003;163:2301–2305. doi: 10.1001/archinte.163.19.2301.
72. Sandhu RK, Jimenez MC, Chiuve SE, Fitzgerald KC, Kenfield SA, Tedrow UB, Albert CM. Smoking, smoking cessation, and risk of sudden cardiac death in women. *Circ Arrhythm Electrophysiol*. 2012;5:1091–1097. doi: 10.1161/CIRCEP.112.975219.
73. Rahimi K, Majoni W, Merhi A, Emberson J. Effect of statins on ventricular tachyarrhythmia, cardiac arrest, and sudden cardiac death: a meta-analysis of published and unpublished evidence from randomized trials. *Eur Heart J*. 2012;33:1571–1581. doi: 10.1093/eurheartj/ehs005.
74. Friedlander Y, Siscovick DS, Weinmann S, Austin MA, Psaty BM, Lemaitre RN, Arbogast P, Raghunathan TE, Cobb LA. Family history as a risk factor for primary cardiac arrest. *Circulation*. 1998;97:155–160.
75. Dekker LR, Bezzina CR, Henriques JP, Tanck MW, Koch KT, Alings MW, Arnold AE, de Boer MJ, Gorgels AP, Michels HR, Verkerk A, Verheugt FW, Zijlstra F, Wilde AA. Familial sudden death is an important risk factor for primary ventricular fibrillation: a case-control study in acute myocardial infarction patients. *Circulation*. 2006;114:1140–1145. doi: 10.1161/CIRCULATIONAHA.105.606145.
76. Kaikkonen KS, Kortelainen ML, Linna E, Huikuri HV. Family history and the risk of sudden cardiac death as a manifestation of an acute coronary event. *Circulation*. 2006;114:1462–1467. doi: 10.1161/CIRCULATIONAHA.106.624593.
77. Jabbari R, Engstrom T, Glinge C, et al. Incidence and risk factors of ventricular fibrillation before primary angioplasty in patients with first ST-elevation myocardial infarction: A nationwide study in Denmrk. *J Am Heart Assoc*. 2014. In press.
78. Bezzina CR, Pazoki R, Bardai A, et al. Genome-wide association study identifies a susceptibility locus at 21q21 for ventricular fibrillation

in acute myocardial infarction. *Nat Genet.* 2010;42:688–691. doi: 10.1038/ng.623.
79. Arking DE, Junttila MJ, Goyette P, et al. Identification of a sudden cardiac death susceptibility locus at 2q24.2 through genome-wide association in European ancestry individuals. *PLoS Genet.* 2011;7:e1002158. doi: 10.1371/journal.pgen.1002158.
80. Siscovick DS, Raghunathan TE, King I, Weinmann S, Wicklund KG, Albright J, Bovbjerg V, Arbogast P, Smith H, Kushi LH. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA.* 1995;274:1363–1367.
81. Albert CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN, Manson JE. Fish consumption and risk of sudden cardiac death. *JAMA.* 1998;279:23–28.
82. Mozaffarian D, Lemaitre RN, Kuller LH, Burke GL, Tracy RP, Siscovick DS; Cardiovascular Health Study. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study. *Circulation.* 2003;107:1372–1377.
83. Chiuve SE, Rimm EB, Sandhu RK, Bernstein AM, Rexrode KM, Manson JE, Willett WC, Albert CM. Dietary fat quality and risk of sudden cardiac death in women. *Am J Clin Nutr.* 2012;96:498–507.
84. Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC, Ma J. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med.* 2002;346:1113–1118. doi: 10.1056/NEJMoa012918.
85. Leaf A, Kang JX, Xiao YF, Billman GE. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. *Circulation.* 2003;107:2646–2652. doi: 10.1161/01.CIR.0000069566.78305.33.
86. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin e after myocardial infarction: Results of the gissi-prevenzione trial. Gruppo italiano per lo studio della sopravvivenza nell'infarto miocardico. *Lancet.* 1999;354:447–455.
87. Kromhout D, Giltay EJ, Geleijnse JM; Alpha Omega Trial Group. n-3 fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med.* 2010;363:2015–2026. doi: 10.1056/NEJMoa1003603.
88. Rauch B, Schiele R, Schneider S, Diller F, Victor N, Gohlke H, Gottwik M, Steinbeck G, Del Castillo U, Sack R, Worth H, Katus H, Spitzer W, Sabin G, Senges J; OMEGA Study Group. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. *Circulation.* 2010;122:2152–2159. doi: 10.1161/CIRCULATIONAHA.110.948562.
89. Chiuve SE, Korngold EC, Januzzi JL Jr, Gantzer ML, Albert CM. Plasma and dietary magnesium and risk of sudden cardiac death in women. *Am J Clin Nutr.* 2011;93:253–260. doi: 10.3945/ajcn.110.002253.
90. Peacock JM, Ohira T, Post W, Sotoodehnia N, Rosamond W, Folsom AR. Serum magnesium and risk of sudden cardiac death in the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J.* 2010;160:464–470. doi: 10.1016/j.ahj.2010.06.012.
91. Zarraga IG, Schwarz ER. Impact of dietary patterns and interventions on cardiovascular health. *Circulation.* 2006;114:961–973. doi: 10.1161/CIRCULATIONAHA.105.603910.
92. Chiuve SE, Fung TT, Rexrode KM, Spiegelman D, Manson JE, Stampfer MJ, Albert CM. Adherence to a low-risk, healthy lifestyle and risk of sudden cardiac death among women. *JAMA.* 2011;306:62–69. doi: 10.1001/jama.2011.907.
93. Bertoia ML, Triche EW, Michaud DS, Baylin A, Hogan JW, Neuhouser ML, Tinker LF, Van Horn L, Waring ME, Li W, Shikany JM, Eaton CB. Mediterranean and Dietary Approaches to Stop Hypertension dietary patterns and risk of sudden cardiac death in postmenopausal women. *Am J Clin Nutr.* 2014;99:344–351. doi: 10.3945/ajcn.112.056135.
94. Albert CM, Manson JE, Cook NR, Ajani UA, Gaziano JM, Hennekens CH. Moderate alcohol consumption and the risk of sudden cardiac death among US male physicians. *Circulation.* 1999;100:944–950.
95. Chiuve SE, Rimm EB, Mukamal KJ, Rexrode KM, Stampfer MJ, Manson JE, Albert CM. Light-to-moderate alcohol consumption and risk of sudden cardiac death in women. *Heart Rhythm.* 2010;7:1374–1380. doi: 10.1016/j.hrthm.2010.05.035.
96. Bertoia ML, Triche EW, Michaud DS, Baylin A, Hogan JW, Neuhouser ML, Freiberg MS, Allison MA, Safford MM, Li W, Mossavar-Rahmani Y, Rosal MC, Eaton CB. Long-term alcohol and caffeine intake and risk of sudden cardiac death in women. *Am J Clin Nutr.* 2013;97:1356–1363. doi: 10.3945/ajcn.112.044248.
97. Wannamethee G, Shaper AG. Alcohol and sudden cardiac death. *Br Heart J.* 1992;68:443–448.
98. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. *N Engl J Med.* 1993;329:1829–1834. doi: 10.1056/NEJM199312163292501.
99. Marijon E, Le Heuzey JY, Connolly S, Yang S, Pogue J, Brueckmann M, Eikelboom J, Themeles E, Ezekowitz M, Wallentin L, Yusuf S; RELY Investigators. Causes of death and influencing factors in patients with atrial fibrillation: a competing-risk analysis from the randomized evaluation of long-term anticoagulant therapy study. *Circulation.* 2013;128:2192–2201. doi: 10.1161/CIRCULATIONAHA.112.000491.
100. Chen LY, Sotoodehnia N, Bůžková P, Lopez FL, Yee LM, Heckbert SR, Prineas R, Soliman EZ, Adabag S, Konety S, Folsom AR, Siscovick D, Alonso A. Atrial fibrillation and the risk of sudden cardiac death: the atherosclerosis risk in communities study and cardiovascular health study. *JAMA Intern Med.* 2013;173:29–35. doi: 10.1001/2013.jamainternmed.744.
101. Bardai A, Blom MT, van Hoeijen DA, van Deutekom HW, Brouwer HJ, Tan HL. Atrial fibrillation is an independent risk factor for ventricular fibrillation: a large-scale population-based case-control study. *Circ Arrhythm Electrophysiol.* 2014;7:1033–1039. doi: 10.1161/CIRCEP.114.002094.
102. Reinier K, Marijon E, Uy-Evanado A, Teodorescu C, Narayanan K, Chugh H, Gunson K, Jui J, Chugh SS. The association between atrial fibrillation and sudden cardiac death: the relevance of heart failure. *JACC Heart Fail.* 2014;2:221–227. doi: 10.1016/j.jchf.2013.12.006.
103. Collins AJ, Foley RN, Chavers B, et al. United states renal data system 2011 annual data report: Atlas of chronic kidney disease & end-stage renal disease in the United States. *Am J Kidney Dis.* 2012;59:A7, e1–420.
104. Deo R, Sotoodehnia N, Katz R, Sarnak MJ, Fried LF, Chonchol M, Kestenbaum B, Psaty BM, Siscovick DS, Shlipak MG. Cystatin C and sudden cardiac death risk in the elderly. *Circ Cardiovasc Qual Outcomes.* 2010;3:159–164. doi: 10.1161/CIRCOUTCOMES.109.875369.
105. Gami AS, Olson EJ, Shen WK, Wright RS, Ballman KV, Hodge DO, Herges RM, Howard DE, Somers VK. Obstructive sleep apnea and the risk of sudden cardiac death: a longitudinal study of 10,701 adults. *J Am Coll Cardiol.* 2013;62:610–616. doi: 10.1016/j.jacc.2013.04.080.
106. Bardai A, Blom MT, van Noord C, Verhamme KM, Sturkenboom MC, Tan HL. Sudden cardiac death is associated both with epilepsy and with use of antiepileptic medications. *Heart.* 2015;101:17–22. doi: 10.1136/heartjnl-2014-305664.
107. Cohen MC, Rohtla KM, Lavery CE, Muller JE, Mittleman MA. Meta-analysis of the morning excess of acute myocardial infarction and sudden cardiac death. *Am J Cardiol.* 1997;79:1512–1516.
108. Willich SN, Goldberg RJ, Maclure M, Perriello L, Muller JE. Increased onset of sudden cardiac death in the first three hours after awakening. *Am J Cardiol.* 1992;70:65–68.
109. Peckova M, Fahrenbruch CE, Cobb LA, Hallstrom AP. Weekly and seasonal variation in the incidence of cardiac arrests. *Am Heart J.* 1999;137:512–515.
110. Arntz HR, Willich SN, Schreiber C, Brüggemann T, Stern R, Schultheiss HP. Diurnal, weekly and seasonal variation of sudden death. Population-based analysis of 24,061 consecutive cases. *Eur Heart J.* 2000;21:315–320. doi: 10.1053/euhj.1999.1739.
111. Peters RW. Propranolol and the morning increase in sudden cardiac death: (the beta-blocker heart attack trial experience). *Am J Cardiol.* 1990;66:57G–59G.
112. Gerber Y, Jacobsen SJ, Killian JM, Weston SA, Roger VL. Seasonality and daily weather conditions in relation to myocardial infarction and sudden cardiac death in Olmsted County, Minnesota, 1979 to 2002. *J Am Coll Cardiol.* 2006;48:287–292. doi: 10.1016/j.jacc.2006.02.065.
113. Chung FP, Li HR, Chong E, Pan CH, Lin YJ, Chang SL, Lo LW, Hu YF, Tuan TC, Chao TF, Liao JN, Lin WY, Shaw KP, Chen SA. Seasonal variation in the frequency of sudden cardiac death and ventricular tachyarrhythmia in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy: the effect of meteorological factors. *Heart Rhythm.* 2013;10:1859–1866. doi: 10.1016/j.hrthm.2013.09.069.
114. Takigawa M, Noda T, Shimizu W, Miyamoto K, Okamura H, Satomi K, Suyama K, Aihara N, Kamakura S, Kurita T. Seasonal and circadian distributions of ventricular fibrillation in patients with Brugada syndrome. *Heart Rhythm.* 2008;5:1523–1527. doi: 10.1016/j.hrthm.2008.08.022.
115. Empana JP, Sauval P, Ducimetiere P, Tafflet M, Carli P, Jouven X. Increase in out-of-hospital cardiac arrest attended by the medical mobile intensive care units, but not myocardial infarction, during the 2003 heat wave in Paris, France. *Crit Care Med.* 2009;37:3079–3084. doi: 10.1097/CCM.0b013e3181b0868f.

116. Siscovick DS, Weiss NS, Fletcher RH, Lasky T. The incidence of primary cardiac arrest during vigorous exercise. *N Engl J Med*. 1984;311:874–877. doi: 10.1056/NEJM198410043111402.
117. Lemaitre RN, Siscovick DS, Raghunathan TE, Weinmann S, Arbogast P, Lin DY. Leisure-time physical activity and the risk of primary cardiac arrest. *Arch Intern Med*. 1999;159:686–690.
118. Multiple Risk Factor Intervention Trial Research Group. Risk factor changes and mortality results. *JAMA*. 1982;248:1465–1477.
119. Whang W, Manson JE, Hu FB, Chae CU, Rexrode KM, Willett WC, Stampfer MJ, Albert CM. Physical exertion, exercise, and sudden cardiac death in women. *JAMA*. 2006;295:1399–1403. doi: 10.1001/jama.295.12.1399.
120. Kannel WB. Exercise and sudden death. *JAMA*. 1982;248:3143–3144.
121. Albert CM, Mittleman MA, Chae CU, Lee IM, Hennekens CH, Manson JE. Triggering of sudden death from cardiac causes by vigorous exertion. *N Engl J Med*. 2000;343:1355–1361. doi: 10.1056/NEJM200011093431902.
122. Berdowski J, de Beus MF, Blom M, Bardai A, Bots ML, Doevendans PA, Grobbee DE, Tan HL, Tijssen JG, Koster RW, Mosterd A. Exercise-related out-of-hospital cardiac arrest in the general population: incidence and prognosis. *Eur Heart J*. 2013;34:3616–3623. doi: 10.1093/eurheartj/eht401.
123. Marijon E, Tafflet M, Celermajer DS, Dumas F, Perier MC, Mustafic H, Toussaint JF, Desnos M, Rieu M, Benameur N, Le Heuzey JY, Empana JP, Jouven X. Sports-related sudden death in the general population. *Circulation*. 2011;124:672–681. doi: 10.1161/CIRCULATIONAHA.110.008979.
124. Kim JH, Malhotra R, Chiampas G, d'Hemecourt P, Troyanos C, Cianca J, Smith RN, Wang TJ, Roberts WO, Thompson PD, Baggish AL; Race Associated Cardiac Arrest Event Registry (RACER) Study Group. Cardiac arrest during long-distance running races. *N Engl J Med*. 2012;366:130–140. doi: 10.1056/NEJMoa1106468.
125. Kawachi I, Colditz GA, Ascherio A, Rimm EB, Giovannucci E, Stampfer MJ, Willett WC. Prospective study of phobic anxiety and risk of coronary heart disease in men. *Circulation*. 1994;89:1992–1997.
126. Albert CM, Chae CU, Rexrode KM, Manson JE, Kawachi I. Phobic anxiety and risk of coronary heart disease and sudden cardiac death among women. *Circulation*. 2005;111:480–487. doi: 10.1161/01.CIR.0000153813.64165.5D.
127. Empana JP, Jouven X, Lemaitre RN, Sotoodehnia N, Rea T, Raghunathan TE, Simon G, Siscovick DS. Clinical depression and risk of out-of-hospital cardiac arrest. *Arch Intern Med*. 2006;166:195–200. doi: 10.1001/archinte.166.2.195.
128. Whang W, Kubzansky LD, Kawachi I, Rexrode KM, Kroenke CH, Glynn RJ, Garan H, Albert CM. Depression and risk of sudden cardiac death and coronary heart disease in women: results from the Nurses' Health Study. *J Am Coll Cardiol*. 2009;53:950–958. doi: 10.1016/j.jacc.2008.10.060.
129. Davidson M. Risk of cardiovascular disease and sudden death in schizophrenia. *J Clin Psychiatry*. 2002;63(Suppl 9):5–11.
130. Weeke P, Jensen A, Folke F, et al. Antidepressant use and risk of out-of-hospital cardiac arrest: a nationwide case-time-control study. *Clin Pharmacol Ther*. 2012;92:72–79. doi: 10.1038/clpt.2011.368.
131. Ray WA, Chung CP, Murray KT, Hall K, Stein CM. Atypical antipsychotic drugs and the risk of sudden cardiac death. *N Engl J Med*. 2009;360:225–235. doi: 10.1056/NEJMoa0806994.
132. Leor J, Poole WK, Kloner RA. Sudden cardiac death triggered by an earthquake. *N Engl J Med*. 1996;334:413–419. doi: 10.1056/NEJM199602153340701.
133. Meisel SR, Kutz I, Dayan KI, Pauzner H, Chetboun I, Arbel Y, David D. Effect of Iraqi missile war on incidence of acute myocardial infarction and sudden death in Israeli civilians. *Lancet*. 1991;338:660–661.
134. Kitamura T, Kiyohara K, Iwami T. The great east Japan earthquake and out-of-hospital cardiac arrest. *N Engl J Med*. 2013;369:2165–2167. doi: 10.1056/NEJMc1306058.
135. Niiyama M, Tanaka F, Nakajima S, Itoh T, Matsumoto T, Kawakami M, Naganuma Y, Omama S, Komatsu T, Onoda T, Sakata K, Ichikawa T, Nakamura M. Population-based incidence of sudden cardiac and unexpected death before and after the 2011 earthquake and tsunami in Iwate, northeast Japan. *J Am Heart Assoc*. 2014;3: e000798. doi: 10.1161/JAHA.114.000798.
136. Teng TH, Williams TA, Bremner A, Tohira H, Franklin P, Tonkin A, Jacobs I, Finn J. A systematic review of air pollution and incidence of out-of-hospital cardiac arrest. *J Epidemiol Community Health*. 2014;68:37–43. doi: 10.1136/jech-2013-203116.
137. Forastiere F, Stafoggia M, Picciotto S, Bellander T, D'Ippoliti D, Lanki T, von Klot S, Nyberg F, Paatero P, Peters A, Pekkanen J, Sunyer J, Perucci CA. A case-crossover analysis of out-of-hospital coronary deaths and air pollution in Rome, Italy. *Am J Respir Crit Care Med*. 2005;172:1549–1555. doi: 10.1164/rccm.200412-1726OC.
138. Raza A, Bellander T, Bero-Bedada G, Dahlquist M, Hollenberg J, Jonsson M, Lind T, Rosenqvist M, Svensson L, Ljungman PL. Short-term effects of air pollution on out-of-hospital cardiac arrest in Stockholm. *Eur Heart J*. 2014;35:861–868. doi: 10.1093/eurheartj/eht489.
139. Silverman RA, Ito K, Freese J, Kaufman BJ, De Claro D, Braun J, Prezant DJ. Association of ambient fine particles with out-of-hospital cardiac arrests in New York City. *Am J Epidemiol*. 2010;172:917–923. doi: 10.1093/aje/kwq217.
140. Ensor KB, Raun LH, Persse D. A case-crossover analysis of out-of-hospital cardiac arrest and air pollution. *Circulation*. 2013;127:1192–1199. doi: 10.1161/CIRCULATIONAHA.113.000027.
141. Dennekamp M, Akram M, Abramson MJ, Tonkin A, Sim MR, Fridman M, Erbas B. Outdoor air pollution as a trigger for out-of-hospital cardiac arrests. *Epidemiology*. 2010;21:494–500. doi: 10.1097/EDE.0b013e3181e093db.
142. Levy D, Sheppard L, Checkoway H, Kaufman J, Lumley T, Koenig J, Siscovick D. A case-crossover analysis of particulate matter air pollution and out-of-hospital primary cardiac arrest. *Epidemiology*. 2001;12:193–199.
143. Wichmann J, Folke F, Torp-Pedersen C, Lippert F, Ketzel M, Ellermann T, Loft S. Out-of-hospital cardiac arrests and outdoor air pollution exposure in Copenhagen, Denmark. *PLoS One*. 2013;8:e53684. doi: 10.1371/journal.pone.0053684.
144. Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL, Kaufman JD. Long-term exposure to air pollution and incidence of cardiovascular events in women. *N Engl J Med*. 2007;356:447–458. doi: 10.1056/NEJMoa054409.
145. Puett RC, Schwartz J, Hart JE, Yanosky JD, Speizer FE, Suh H, Paciorek CJ, Neas LM, Laden F. Chronic particulate exposure, mortality, and coronary heart disease in the nurses' health study. *Am J Epidemiol*. 2008;168:1161–1168. doi: 10.1093/aje/kwn232.
146. Hart JE, Chiuve SE, Laden F, Albert CM. Roadway proximity and risk of sudden cardiac death in women. *Circulation*. 2014;130:1474–1482. doi: 10.1161/CIRCULATIONAHA.114.011489.
147. Kannel WB, Cupples LA, D'Agostino RB. Sudden death risk in overt coronary heart disease: the Framingham Study. *Am Heart J*. 1987;113:799–804.
148. Solomon SD, Zelenkofske S, McMurray JJ, Finn PV, Velazquez E, Ertl G, Harsanyi A, Rouleau JL, Maggioni A, Kober L, White H, Van de Werf F, Pieper K, Califf RM, Pfeffer MA; Valsartan in Acute Myocardial Infarction Trial (VALIANT) Investigators. Sudden death in patients with myocardial infarction and left ventricular dysfunction, heart failure, or both. *N Engl J Med*. 2005;352:2581–2588. doi: 10.1056/NEJMoa043938.
149. Adabag AS, Therneau TM, Gersh BJ, Weston SA, Roger VL. Sudden death after myocardial infarction. *JAMA*. 2008;300:2022–2029. doi: 10.1001/jama.2008.553.
150. Huikuri HV, Tapanainen JM, Lindgren K, Raatikainen P, Mäkikallio TH, Juhani Airaksinen KE, Myerburg RJ. Prediction of sudden cardiac death after myocardial infarction in the beta-blocking era. *J Am Coll Cardiol*. 2003;42:652–658.
151. ADELSON L, HOFFMAN W. Sudden death from coronary disease related to a lethal mechanism arising independently of vascular occlusion or myocardial damage. *JAMA*. 1961;176:129–135.
152. Farb A, Tang AL, Burke AP, Sessums L, Liang Y, Virmani R. Sudden coronary death. Frequency of active coronary lesions, inactive coronary lesions, and myocardial infarction. *Circulation*. 1995;92:1701–1709.
153. Davies MJ, Thomas A. Thrombosis and acute coronary-artery lesions in sudden cardiac ischemic death. *N Engl J Med*. 1984;310:1137–1140. doi: 10.1056/NEJM198405033101801.
154. Kramer MC, Rittersma SZ, de Winter RJ, Ladich ER, Fowler DR, Liang YH, Kutys R, Carter-Monroe N, Kolodgie FD, van der Wal AC, Virmani R. Relationship of thrombus healing to underlying plaque morphology in sudden coronary death. *J Am Coll Cardiol*. 2010;55:122–132. doi: 10.1016/j.jacc.2009.09.007.
155. Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med*. 1997;336:1276–1282. doi: 10.1056/NEJM199705013361802.
156. Burke AP, Farb A, Malcom GT, Liang Y, Smialek J, Virmani R. Effect of risk factors on the mechanism of acute thrombosis and sudden coronary death in women. *Circulation*. 1998;97:2110–2116.

157. Pouleur AC, Barkoudah E, Uno H, et al; VALIANT Investigators. Pathogenesis of sudden unexpected death in a clinical trial of patients with myocardial infarction and left ventricular dysfunction, heart failure, or both. *Circulation*. 2010;122:597–602. doi: 10.1161/CIRCULATIONAHA.110.940619.
158. Stevenson WG, Stevenson LW, Middlekauff HR, Saxon LA. Sudden death prevention in patients with advanced ventricular dysfunction. *Circulation*. 1993;88:2953–2961.
159. Bigger JT Jr, Fleiss JL, Kleiger R, Miller JP, Rolnitzky LM. The relationships among ventricular arrhythmias, left ventricular dysfunction, and mortality in the 2 years after myocardial infarction. *Circulation*. 1984;69:250–258.
160. Mäkikallio TH, Barthel P, Schneider R, Bauer A, Tapanainen JM, Tulppo MP, Perkiömäki JS, Schmidt G, Huikuri HV. Frequency of sudden cardiac death among acute myocardial infarction survivors with optimized medical and revascularization therapy. *Am J Cardiol*. 2006;97:480–484. doi: 10.1016/j.amjcard.2005.09.077.
161. Buxton AE. Risk stratification for sudden death in patients with coronary artery disease. *Heart Rhythm*. 2009;6:836–847. doi: 10.1016/j.hrthm.2009.02.016.
162. Greenberg H, Case RB, Moss AJ, Brown MW, Carroll ER, Andrews ML; MADIT-II Investigators. Analysis of mortality events in the Multicenter Automatic Defibrillator Implantation Trial (MADIT-II). *J Am Coll Cardiol*. 2004;43:1459–1465. doi: 10.1016/j.jacc.2003.11.038.
163. Moss AJ, Zareba W, Hall WJ, Klein H, Wilber DJ, Cannom DS, Daubert JP, Higgins SL, Brown MW, Andrews ML; Multicenter Automatic Defibrillator Implantation Trial II Investigators. Prophylactic implantation of a defibrillator in patients with myocardial infarction and reduced ejection fraction. *N Engl J Med*. 2002;346:877–883. doi: 10.1056/NEJMoa013474.
164. Packer DL, Prutkin JM, Hellkamp AS, et al. Impact of implantable cardioverter-defibrillator, amiodarone, and placebo on the mode of death in stable patients with heart failure: analysis from the sudden cardiac death in heart failure trial. *Circulation*. 2009;120:2170–2176. doi: 10.1161/CIRCULATIONAHA.109.853689.
165. Epstein AE, DiMarco JP, Ellenbogen KA, et al; American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the ACC/AHA/NASPE 2002 Guideline Update for Implantation of Cardiac Pacemakers and Antiarrhythmia Devices); American Association for Thoracic Surgery; Society of Thoracic Surgeons. ACC/AHA/HRS 2008 Guidelines for Device-Based Therapy of Cardiac Rhythm Abnormalities: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the ACC/AHA/NASPE 2002 Guideline Update for Implantation of Cardiac Pacemakers and Antiarrhythmia Devices): developed in collaboration with the American Association for Thoracic Surgery and Society of Thoracic Surgeons. *Circulation*. 2008;117:e350–e408. doi: 10.1161/CIRCUALTIONAHA.108.189742.
166. Hohnloser SH, Kuck KH, Dorian P, Roberts RS, Hampton JR, Hatala R, Fain E, Gent M, Connolly SJ; DINAMIT Investigators. Prophylactic use of an implantable cardioverter-defibrillator after acute myocardial infarction. *N Engl J Med*. 2004;351:2481–2488. doi: 10.1056/NEJMoa041489.
167. Steinbeck G, Andresen D, Seidl K, Brachmann J, Hoffmann E, Wojciechowski D, Kornacewicz-Jach Z, Sredniawa B, Lupkovics G, Hofgärtner F, Lubinski A, Rosenqvist M, Habets A, Wegscheider K, Senges J; IRIS Investigators. Defibrillator implantation early after myocardial infarction. *N Engl J Med*. 2009;361:1427–1436. doi: 10.1056/NEJMoa0901889.
168. Buxton AE. Should everyone with an ejection fraction less than or equal to 30% receive an implantable cardioverter-defibrillator? Not everyone with an ejection fraction < or = 30% should receive an implantable cardioverter-defibrillator. *Circulation*. 2005;111:2537–49; discussion 2537. doi: 10.1161/01.CIR.0000165057.88551.2C.
169. Cheng A, Zhang Y, Blasco-Colmenares E, Dalal D, Butcher B, Norgard S, Eldadah Z, Ellenbogen KA, Dickfeld T, Spragg DD, Marine JE, Guallar E, Tomaselli GF. Protein biomarkers identify patients unlikely to benefit from primary prevention implantable cardioverter defibrillators: findings from the Prospective Observatoinal Study of Implantable Cardioverter Defibrillators (PROSE-ICD). *Circ Arrhythm Electrophysiol*. 2014;7:1084–1091.
170. Stecker EC, Vickers C, Waltz J, Socoteanu C, John BT, Mariani R, McAnulty JH, Gunson K, Jui J, Chugh SS. Population-based analysis of sudden cardiac death with and without left ventricular systolic dysfunction: two-year findings from the Oregon Sudden Unexpected Death Study. *J Am Coll Cardiol*. 2006;47:1161–1166. doi: 10.1016/j.jacc.2005.11.045.
171. Buxton AE, Lee KL, Fisher JD, Josephson ME, Prystowsky EN, Hafley G. A randomized study of the prevention of sudden death in patients with coronary artery disease. Multicenter Unsustained Tachycardia Trial Investigators. *N Engl J Med*. 1999;341:1882–1890. doi: 10.1056/NEJM199912163412503.
172. Lee DC, Goldberger JJ. CMR for sudden cardiac death risk stratification: are we there yet? *JACC Cardiovasc Imaging*. 2013;6:345–348. doi: 10.1016/j.jcmg.2012.12.006.
173. Exner DV, Kavanagh KM, Slawnych MP, et al; REFINE Investigators. Noninvasive risk assessment early after a myocardial infarction the REFINE study. *J Am Coll Cardiol*. 2007;50:2275–2284. doi: 10.1016/j.jacc.2007.08.042.
174. Zaman S, Narayan A, Thiagalingam A, Sivagangabalan G, Thomas S, Ross DL, Kovoor P. Long-term arrhythmia-free survival in patients with severe left ventricular dysfunction and no inducible ventricular tachycardia after myocardial infarction. *Circulation*. 2014;129:848–854. doi: 10.1161/CIRCULATIONAHA.113.005146.
175. Zile MR, Gaasch WH, Anand IS, et al; I-Preserve Investigators. Mode of death in patients with heart failure and a preserved ejection fraction: results from the Irbesartan in Heart Failure With Preserved Ejection Fraction Study (I-Preserve) trial. *Circulation*. 2010;121:1393–1405. doi: 10.1161/CIRCULATIONAHA.109.909614.
176. Chan MM, Lam CS. How do patients with heart failure with preserved ejection fraction die? *Eur J Heart Fail*. 2013;15:604–613. doi: 10.1093/eurjhf/hft062.
177. Solomon SD, Wang D, Finn P, Skali H, Zornoff L, McMurray JJ, Swedberg K, Yusuf S, Granger CB, Michelson EL, Pocock S, Pfeffer MA. Effect of candesartan on cause-specific mortality in heart failure patients: the Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity (CHARM) program. *Circulation*. 2004;110:2180–2183. doi: 10.1161/01.CIR.0000144474.65922.AA.
178. Al-Khatib SM, Shaw LK, O'Connor C, Kong M, Califf RM. Incidence and predictors of sudden cardiac death in patients with diastolic heart failure. *J Cardiovasc Electrophysiol*. 2007;18:1231–1235. doi: 10.1111/j.1540-8167.2007.00957.x.
179. Adabag S, Rector TS, Anand IS, McMurray JJ, Zile M, Komajda M, McKelvie RS, Massie B, Carson PE. A prediction model for sudden cardiac death in patients with heart failure and preserved ejection fraction. *Eur J Heart Fail*. 2014;16:1175–1182. doi: 10.1002/ejhf.172.
180. Corrado D, Basso C, Thiene G. Sudden cardiac death in young people with apparently normal heart. *Cardiovasc Res*. 2001;50:399–408.
181. Puranik R, Chow CK, Duflou JA, Kilborn MJ, McGuire MA. Sudden death in the young. *Heart Rhythm*. 2005;2:1277–1282. doi: 10.1016/j.hrthm.2005.09.008.
182. Ikeda T, Yusu S, Nakamura K, Yoshino H. Risk stratification for sudden cardiac death. *Circ J*. 2007;71(Suppl A):A106–A114.
183. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB; American Heart Association; Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; Council on Epidemiology and Prevention. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation*. 2006;113:1807–1816. doi: 10.1161/CIRCULATIONAHA.106.174287.
184. Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J*. 2008;29:270–276. doi: 10.1093/eurheartj/ehm342.
185. Lakdawala NK, Winterfield JR, Funke BH. Dilated cardiomyopathy. *Circ Arrhythm Electrophysiol*. 2013;6:228–237. doi: 10.1161/CIRCEP.111.962050.
186. Epstein AE, DiMarco JP, Ellenbogen KA, et al; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines; Heart Rhythm Society. 2012 ACCF/AHA/HRS focused update incorporated into the ACCF/AHA/HRS 2008 guidelines for device-based therapy of cardiac rhythm abnormalities: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Heart

Rhythm Society. *Circulation*. 2013;127:e283–e352. doi: 10.1161/CIR.0b013e318276ce9b.
187. Bardy GH, Lee KL, Mark DB, et al; Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT) Investigators. Amiodarone or an implantable cardioverter-defibrillator for congestive heart failure. *N Engl J Med*. 2005;352:225–237. doi: 10.1056/NEJMoa043399.
188. Kadish A, Dyer A, Daubert JP, Quigg R, Estes NA, Anderson KP, Calkins H, Hoch D, Goldberger J, Shalaby A, Sanders WE, Schaechter A, Levine JH; Defibrillators in Non-Ischemic Cardiomyopathy Treatment Evaluation (DEFINITE) Investigators. Prophylactic defibrillator implantation in patients with nonischemic dilated cardiomyopathy. *N Engl J Med*. 2004;350:2151–2158. doi: 10.1056/NEJMoa033088.
189. Theuns DA, Smith T, Hunink MG, Bardy GH, Jordaens L. Effectiveness of prophylactic implantation of cardioverter-defibrillators without cardiac resynchronization therapy in patients with ischaemic or non-ischaemic heart disease: a systematic review and meta-analysis. *Europace*. 2010;12:1564–1570. doi: 10.1093/europace/euq329.
190. Goldberger JJ, Subačius H, Patel T, Cunnane R, Kadish AH. Sudden cardiac death risk stratification in patients with nonischemic dilated cardiomyopathy. *J Am Coll Cardiol*. 2014;63:1879–1889. doi: 10.1016/j.jacc.2013.12.021.
191. Gulati A, Jabbour A, Ismail TF, et al. Association of fibrosis with mortality and sudden cardiac death in patients with nonischemic dilated cardiomyopathy. *JAMA*. 2013;309:896–908. doi: 10.1001/jama.2013.1363.
192. Maron BJ, Ommen SR, Semsarian C, Spirito P, Olivotto I, Maron MS. Hypertrophic cardiomyopathy: present and future, with translation into contemporary cardiovascular medicine. *J Am Coll Cardiol*. 2014;64:83–99. doi: 10.1016/j.jacc.2014.05.003.
193. Elliott PM, Anastasakis A, Borger MA, et al. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: The task force for the diagnosis and management of hypertrophic cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J*. 2014;35:2733–2779.
194. Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, Naidu SS, Nishimura RA, Ommen SR, Rakowski H, Seidman CE, Towbin JA, Udelson JE, Yancy CW; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines; American Association for Thoracic Surgery; American Society of Echocardiography; American Society of Nuclear Cardiology; Heart Failure Society of America; Heart Rhythm Society; Society for Cardiovascular Angiography and Interventions; Society of Thoracic Surgeons. 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2011;124:2761–2796. doi: 10.1161/CIR.0b013e318223e230.
195. O'Mahony C, Jichi F, Pavlou M, Monserrat L, Anastasakis A, Rapezzi C, Biagini E, Gimeno JR, Limongelli G, McKenna WJ, Omar RZ, Elliott PM; Hypertrophic Cardiomyopathy Outcomes Investigators. A novel clinical risk prediction model for sudden cardiac death in hypertrophic cardiomyopathy (HCM risk-SCD). *Eur Heart J*. 2014;35:2010–2020. doi: 10.1093/eurheartj/eht439.
196. Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. *Circulation*. 2010;121:1533–1541. doi: 10.1161/CIRCULATIONAHA.108.840827.
197. Murray B. Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C): a review of molecular and clinical literature. *J Genet Couns*. 2012;21:494–504. doi: 10.1007/s10897-012-9497-7.
198. den Haan AD, Tan BY, Zikusoka MN, Lladó LI, Jain R, Daly A, Tichnell C, James C, Amat-Alarcon N, Abraham T, Russell SD, Bluemke DA, Calkins H, Dalal D, Judge DP. Comprehensive desmosome mutation analysis in north americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circ Cardiovasc Genet*. 2009;2:428–435. doi: 10.1161/CIRCGENETICS.109.858217.
199. Tabib A, Loire R, Chalabreysse L, Meyronnet D, Miras A, Malicier D, Thivolet F, Chevalier P, Bouvagnet P. Circumstances of death and gross and microscopic observations in a series of 200 cases of sudden death associated with arrhythmogenic right ventricular cardiomyopathy and/or dysplasia. *Circulation*. 2003;108:3000–3005. doi: 10.1161/01.CIR.0000108396.65446.21.
200. Quarta G, Muir A, Pantazis A, Syrris P, Gehmlich K, Garcia-Pavia P, Ward D, Sen-Chowdhry S, Elliott PM, McKenna WJ. Familial evaluation in arrhythmogenic right ventricular cardiomyopathy: impact of genetics and revised task force criteria. *Circulation*. 2011;123:2701–2709. doi: 10.1161/CIRCULATIONAHA.110.976936.
201. Corrado D, Leoni L, Link MS, et al. Implantable cardioverter-defibrillator therapy for prevention of sudden death in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Circulation*. 2003;108:3084–3091. doi: 10.1161/01.CIR.0000103130.33451.D2.
202. Hulot JS, Jouven X, Empana JP, Frank R, Fontaine G. Natural history and risk stratification of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation*. 2004;110:1879–1884. doi: 10.1161/01.CIR.0000143375.93288.82.
203. Lemola K, Brunckhorst C, Helfenstein U, Oechslin E, Jenni R, Duru F. Predictors of adverse outcome in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy: long term experience of a tertiary care centre. *Heart*. 2005;91:1167–1172. doi: 10.1136/hrt.2004.038620.
204. Bhonsale A, James CA, Tichnell C, Murray B, Gagarin D, Philips B, Dalal D, Tedford R, Russell SD, Abraham T, Tandri H, Judge DP, Calkins H. Incidence and predictors of implantable cardioverter-defibrillator therapy in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy undergoing implantable cardioverter-defibrillator implantation for primary prevention. *J Am Coll Cardiol*. 2011;58:1485–1496. doi: 10.1016/j.jacc.2011.06.043.
205. Loire R, Tabib A. [Unexpected sudden cardiac death. An evaluation of 1000 autopsies]. *Arch Mal Coeur Vaiss*. 1996;89:13–18.
206. Groves P. Valve disease: Surgery of valve disease: late results and late complications. *Heart*. 2001;86:715–721.
207. Pellikka PA, Sarano ME, Nishimura RA, Malouf JF, Bailey KR, Scott CG, Barnes ME, Tajik AJ. Outcome of 622 adults with asymptomatic, hemodynamically significant aortic stenosis during prolonged follow-up. *Circulation*. 2005;111:3290–3295. doi: 10.1161/CIRCULATIONAHA.104.495903.
208. Bhattacharyya S, Hayward C, Pepper J, Senior R. Risk stratification in asymptomatic severe aortic stenosis: a critical appraisal. *Eur Heart J*. 2012;33:2377–2387. doi: 10.1093/eurheartj/ehs190.
209. Kang DH, Park SJ, Rim JH, Yun SC, Kim DH, Song JM, Choo SJ, Park SW, Song JK, Lee JW, Park PW. Early surgery versus conventional treatment in asymptomatic very severe aortic stenosis. *Circulation*. 2010;121:1502–1509. doi: 10.1161/CIRCULATIONAHA.109.909903.
210. Hayek E, Gring CN, Griffin BP. Mitral valve prolapse. *Lancet*. 2005;365:507–518. doi: 10.1016/S0140-6736(05)17869-6.
211. Sriram CS, Syed FF, Ferguson ME, Johnson JN, Enriquez-Sarano M, Cetta F, Cannon BC, Asirvatham SJ, Ackerman MJ. Malignant bileaflet mitral valve prolapse syndrome in patients with otherwise idiopathic out-of-hospital cardiac arrest. *J Am Coll Cardiol*. 2013;62:222–230. doi: 10.1016/j.jacc.2013.02.060.
212. Wever EF, Robles de Medina EO. Sudden death in patients without structural heart disease. *J Am Coll Cardiol*. 2004;43:1137–1144. doi: 10.1016/j.jacc.2003.10.053.
213. Behr ER, Dalageorgou C, Christiansen M, Syrris P, Hughes S, Tome Esteban MT, Rowland E, Jeffery S, McKenna WJ. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. *Eur Heart J*. 2008;29:1670–1680. doi: 10.1093/eurheartj/ehn219.
214. Papadakis M, Raju H, Behr ER, De Noronha SV, Spath N, Kouloubinis A, Sheppard MN, Sharma S. Sudden cardiac death with autopsy findings of uncertain significance: potential for erroneous interpretation. *Circ Arrhythm Electrophysiol*. 2013;6:588–596. doi: 10.1161/CIRCEP.113.000111.
215. Hofman N, Tan HL, Alders M, Kolder I, de Haij S, Mannens MM, Lombardi MP, Dit Deprez RH, van Langen I, Wilde AA. Yield of molecular and clinical testing for arrhythmia syndromes: report of 15 years' experience. *Circulation*. 2013;128:1513–1521. doi: 10.1161/CIRCULATIONAHA.112.000091.
216. Schwartz PJ, Periti M, Malliani A. The long Q-T syndrome. *Am Heart J*. 1975;89:378–390.
217. Schwartz PJ, Crotti L. QTc behavior during exercise and genetic testing for the long-QT syndrome. *Circulation*. 2011;124:2181–2184. doi: 10.1161/CIRCULATIONAHA.111.062182.
218. Goldenberg I, Moss AJ. Long QT syndrome. *J Am Coll Cardiol*. 2008;51:2291–2300. doi: 10.1016/j.jacc.2008.02.068.
219. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D. Risk stratification in the long-QT syndrome. *N Engl J Med*. 2003;348:1866–1874. doi: 10.1056/NEJMoa022147.
220. Shimizu W, Noda T, Takaki H, et al. Diagnostic value of epinephrine test for genotyping LQT1, LQT2, and LQT3 forms of congenital

long QT syndrome. *Heart Rhythm.* 2004;1:276–283. doi: 10.1016/j.hrthm.2004.04.021.
221. Shimizu W, Noda T, Takaki H, et al. Epinephrine unmasks latent mutation carriers with LQT1 form of congenital long-QT syndrome. *J Am Coll Cardiol.* 2003;41:633–642.
222. Schwartz PJ, Stramba-Badiale M, Crotti L, et al. Prevalence of the congenital long-QT syndrome. *Circulation.* 2009;120:1761–1767. doi: 10.1161/CIRCULATIONAHA.109.863209.
223. Schwartz PJ, Priori SG, Spazzolini C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation.* 2001;103:89–95.
224. Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Robinson JL, Priori SG, Benhorin J, Locati EH, Towbin JA, Keating MT, Lehmann MH, Hall WJ. Influence of genotype on the clinical course of the long-QT syndrome. International Long-QT Syndrome Registry Research Group. *N Engl J Med.* 1998;339:960–965. doi: 10.1056/NEJM199810013391404.
225. Moss AJ, Shimizu W, Wilde AA, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation.* 2007;115:2481–2489. doi: 10.1161/CIRCULATIONAHA.106.665406.
226. Shimizu W, Moss AJ, Wilde AA, et al. Genotype-phenotype aspects of type 2 long QT syndrome. *J Am Coll Cardiol.* 2009;54:2052–2062. doi: 10.1016/j.jacc.2009.08.028.
227. Shimizu W, Horie M, Ohno S, et al. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in the LQT1 form of congenital long QT syndrome: multicenter study in Japan. *J Am Coll Cardiol.* 2004;44:117–125. doi: 10.1016/j.jacc.2004.03.043.
228. Priori SG, Wilde AA, Horie M, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm.* 2013;10:1932–1963. doi: 10.1016/j.hrthm.2013.05.014.
229. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J Am Coll Cardiol.* 1992;20:1391–1396.
230. Gaw AC, Lee B, Gervacio-Domingo G, Antzelevitch C, Divinagracia R, Jocano F Jr. Unraveling the Enigma of Bangungut: Is Sudden Unexplained Nocturnal Death Syndrome (SUNDS) in the Philippines a Disease Allelic to the Brugada Syndrome? *Philipp J Intern Med.* 2011;49:165–176.
231. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, Corrado D, Hauer RN, Kass RS, Nademanee K, Priori SG, Towbin JA; Study Group on the Molecular Basis of Arrhythmias of the European Society of Cardiology. Proposed diagnostic criteria for the Brugada syndrome: consensus report. *Circulation.* 2002;106:2514–2519.
232. Shimizu W, Matsuo K, Kokubo Y, Satomi K, Kurita T, Noda T, Nagaya N, Suyama K, Aihara N, Kamakura S, Inamoto N, Akahoshi M, Tomoike H. Sex hormone and gender difference–role of testosterone on male predominance in Brugada syndrome. *J Cardiovasc Electrophysiol.* 2007;18:415–421. doi: 10.1111/j.1540-8167.2006.00743.x.
233. Sakabe M, Fujiki A, Tani M, Nishida K, Mizumaki K, Inoue H. Proportion and prognosis of healthy people with coved or saddle-back type ST segment elevation in the right precordial leads during 10 years follow-up. *Eur Heart J.* 2003;24:1488–1493.
234. Tsuji H, Sato T, Morisaki K, Iwasaka T. Prognosis of subjects with Brugada-type electrocardiogram in a population of middle-aged Japanese diagnosed during a health examination. *Am J Cardiol.* 2008;102:584–587. doi: 10.1016/j.amjcard.2008.04.066.
235. Gervacio-Domingo G, Isidro J, Tirona J, Gabriel E, David G, Amarillo ML, Morales D, Dans A. The Brugada type 1 electrocardiographic pattern is common among Filipinos. *J Clin Epidemiol.* 2008;61:1067–1072. doi: 10.1016/j.jclinepi.2007.11.009.
236. Ito H, Yano K, Chen R, He Q, Curb JD. The prevalence and prognosis of a Brugada-type electrocardiogram in a population of middle-aged Japanese-American men with follow-up of three decades. *Am J Med Sci.* 2006;331:25–29.
237. Letsas KP, Gavrielatos G, Efremidis M, Kounas SP, Filippatos GS, Sideris A, Kardaras F. Prevalence of Brugada sign in a Greek tertiary hospital population. *Europace.* 2007;9:1077–1080. doi: 10.1093/europace/eum221.
238. Gallagher MM, Forleo GB, Behr ER, Magliano G, De Luca L, Morgia V, De Liberato F, Romeo F. Prevalence and significance of Brugada-type ECG in 12,012 apparently healthy European subjects. *Int J Cardiol.* 2008;130:44–48. doi: 10.1016/j.ijcard.2007.07.159.
239. Pecini R, Cedergreen P, Theilade S, Haunsø S, Theilade J, Jensen GB. The prevalence and relevance of the Brugada-type electrocardiogram in the Danish general population: data from the Copenhagen City Heart Study. *Europace.* 2010;12:982–986. doi: 10.1093/europace/euq077.
240. Patel SS, Anees S, Anees SS, Ferrick KJ. Prevalence of a Brugada pattern electrocardiogram in an urban population in the United States. *Pacing Clin Electrophysiol.* 2009;32:704–708. doi: 10.1111/j.1540-8159.2009.02354.x.
241. Lee C, Soni A, Tate RB, Cuddy TE. The incidence and prognosis of Brugada electrocardiographic pattern in the Manitoba Follow-Up Study. *Can J Cardiol.* 2005;21:1286–1290.
242. Kamakura S, Ohe T, Nakazawa K, et al; Brugada Syndrome Investigators in Japan. Long-term prognosis of probands with Brugada-pattern ST-elevation in leads V1-V3. *Circ Arrhythm Electrophysiol.* 2009;2:495–503. doi: 10.1161/CIRCEP.108.816892.
243. Probst V, Veltmann C, Eckardt L, et al. Long-term prognosis of patients diagnosed with Brugada syndrome: Results from the FINGER Brugada Syndrome Registry. *Circulation.* 2010;121:635–643. doi: 10.1161/CIRCULATIONAHA.109.887026.
244. Delise P, Allocca G, Marras E, Giustetto C, Gaita F, Sciarra L, Calo L, Proclemer A, Marziali M, Rebellato L, Berton G, Coro L, Sitta N. Risk stratification in individuals with the Brugada type 1 ECG pattern without previous cardiac arrest: usefulness of a combined clinical and electrophysiologic approach. *Eur Heart J.* 2011;32:169–176. doi: 10.1093/eurheartj/ehq381.
245. Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, Coumel P. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. *Circulation.* 1995;91:1512–1519.
246. Hayashi M, Denjoy I, Hayashi M, Extramiana F, Maltret A, Roux-Buisson N, Lupoglazoff JM, Klug D, Maury P, Messali A, Guicheney P, Leenhardt A. The role of stress test for predicting genetic mutations and future cardiac events in asymptomatic relatives of catecholaminergic polymorphic ventricular tachycardia probands. *Europace.* 2012;14:1344–1351. doi: 10.1093/europace/eus031.
247. Hayashi M, Denjoy I, Extramiana F, Maltret A, Buisson NR, Lupoglazoff JM, Klug D, Hayashi M, Takatsuki S, Villain E, Kamblock J, Messali A, Guicheney P, Lunardi J, Leenhardt A. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. *Circulation.* 2009;119:2426–2434. doi: 10.1161/CIRCULATIONAHA.108.829267.
248. Priori SG, Napolitano C, Memmi M, Colombi B, Drago F, Gasparini M, DeSimone L, Coltorti F, Bloise R, Keegan R, Cruz Filho FE, Vignati G, Benatar A, DeLogu A. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation.* 2002;106:69–74.
249. Sumitomo N, Harada K, Nagashima M, et al. Catecholaminergic polymorphic ventricular tachycardia: electrocardiographic characteristics and optimal therapeutic strategies to prevent sudden death. *Heart.* 2003;89:66–70.
250. Swan H, Piippo K, Viitasalo M, Heikkilä P, Paavonen T, Kainulainen K, Kere J, Keto P, Kontula K, Toivonen L. Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. *J Am Coll Cardiol.* 1999;34:2035–2042.
251. Watanabe H, Chopra N, Laver D, Hwang HS, Davies SS, Roach DE, Duff HJ, Roden DM, Wilde AA, Knollmann BC. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. *Nat Med.* 2009;15:380–383. doi: 10.1038/nm.1942.
252. FENICHEL NN. A long term study of concave RS-T elevation–a normal variant of the electrocardiogram. *Angiology.* 1962;13:360–366.
253. Klatsky AL, Oehm R, Cooper RA, Udaltsova N, Armstrong MA. The early repolarization normal variant electrocardiogram: correlates and consequences. *Am J Med.* 2003;115:171–177.
254. Haïssaguerre M, Derval N, Sacher F, et al. Sudden cardiac arrest associated with early repolarization. *N Engl J Med.* 2008;358:2016–2023. doi: 10.1056/NEJMoa071968.
255. Rosso R, Kogan E, Belhassen B, Rozovski U, Scheinman MM, Zeltser D, Halkin A, Steinvil A, Heller K, Glikson M, Katz A, Viskin S. J-point elevation in survivors of primary ventricular fibrillation and matched control subjects: incidence and clinical significance. *J Am Coll Cardiol.* 2008;52:1231–1238. doi: 10.1016/j.jacc.2008.07.010.
256. Tikkanen JT, Anttonen O, Junttila MJ, Aro AL, Kerola T, Rissanen HA, Reunanen A, Huikuri HV. Long-term outcome associated with early repolarization on electrocardiography. *N Engl J Med.* 2009;361:2529–2537. doi: 10.1056/NEJMoa0907589.

257. Sinner MF, Reinhard W, Müller M, et al. Association of early repolarization pattern on ECG with risk of cardiac and all-cause mortality: a population-based prospective cohort study (MONICA/KORA). *PLoS Med*. 2010;7:e1000314. doi: 10.1371/journal.pmed.1000314.
258. Olson KA, Viera AJ, Soliman EZ, Crow RS, Rosamond WD. Long-term prognosis associated with J-point elevation in a large middle-aged biracial cohort: the ARIC study. *Eur Heart J*. 2011;32:3098–3106. doi: 10.1093/eurheartj/ehr264.
259. Ilkhanoff L, Soliman EZ, Prineas RJ, Walsh JA 3rd, Ning H, Liu K, Carr JJ, Jacobs DR Jr, Lloyd-Jones DM. Clinical characteristics and outcomes associated with the natural history of early repolarization in a young, biracial cohort followed to middle age: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Circ Arrhythm Electrophysiol*. 2014;7:392–399. doi: 10.1161/CIRCEP.113.000874.
260. Haruta D, Matsuo K, Tsuneto A, Ichimaru S, Hida A, Sera N, Imaizumi M, Nakashima E, Maemura K, Akahoshi M. Incidence and prognostic value of early repolarization pattern in the 12-lead electrocardiogram. *Circulation*. 2011;123:2931–2937. doi: 10.1161/CIRCULATIONAHA.110.006460.
261. Hisamatsu T, Ohkubo T, Miura K, Yamamoto T, Fujiyoshi A, Miyagawa N, Kadota A, Takashima N, Nagasawa SY, Kita Y, Murakami Y, Okayama A, Horie M, Okamura T, Ueshima H; NIPPON DATA90 Research Group. Association between J-point elevation and death from coronary artery disease–15-year follow up of the NIPPON DATA90. *Circ J*. 2013;77:1260–1266.
262. Noseworthy PA, Tikkanen JT, Porthan K, Oikarinen L, Pietilä A, Harald K, Peloso GM, Merchant FM, Jula A, Väänänen H, Hwang SJ, O'Donnell CJ, Salomaa V, Newton-Cheh C, Huikuri HV. The early repolarization pattern in the general population: clinical correlates and heritability. *J Am Coll Cardiol*. 2011;57:2284–2289. doi: 10.1016/j.jacc.2011.04.003.
263. Tikkanen JT, Junttila MJ, Anttonen O, Aro AL, Luttinen S, Kerola T, Sager SJ, Rissanen HA, Myerburg RJ, Reunanen A, Huikuri HV. Early repolarization: electrocardiographic phenotypes associated with favorable long-term outcome. *Circulation*. 2011;123:2666–2673. doi: 10.1161/CIRCULATIONAHA.110.014068.
264. Junttila MJ, Raatikainen MJP, Karjalainen J, Kauma H, Kesaniemi YA, Huikuri HV. Prevalence and prognosis of subjects with Brugada-type ECG pattern in a young and middle-aged Finnish population. *Eur Heart J*. 2004;25:874–878.
265. Sinner MF, Pfeufer A, Perz S, Schulze-Bahr E, Monnig G, Eckardt L, Beckmann BM, Wichmann HE, Breithardt G, Steinbeck G, Fabritz L, Kaab S, Kirchhof P. Spontaneous Brugada electrocardiogram patterns are rare in the German general population: results from the KORA study. *Europace*. 2009;11:1338–1344.
266. Yamakawa Y, Ishikawa T, Uchino K, Mochida Y, Ebina T, Sumita S, Kobayashi T, Matsushita K, Matsumoto K, Ohkusu Y, Nishizawa T, Takigiku K, Iwamoto M, Kimura K, Umemura S. Prevalence of right bundle-branch block and right precordial ST-segment elevation (Brugada-type electrocardiogram) in Japanese children. *Circ J*. 2004;68:275–279.
267. Oe H, Takagi M, Tanaka A, Namba M, Nishibori Y, Nishida Y, Kawarabayashi T, Yoshiyama M, Nishimoto M, Tanaka K, Yoshikawa J. Prevalence and clinical course of the juveniles with Brugada-type ECG in Japanese population. *Pacing Clin Electrophysiol*. 2005;28:549–554.
268. Rollin A, Maury P, Bongard V, Sacher F, Delay M, Duparc A, Mondoly P, Carrié D, Ferrières J, Ruidavets JB. Prevalence, prognosis, and identification of the malignant form of early repolarization pattern in a population-based study. *Am J Cardiol*. 2012;110:1302–1308.
269. Uberoi A, MD, Jain NA, Perez M, Weinkopff A, Ashley E, Hadley D, Turakhia MP, Froelicher V. Early repolarization in an ambulatory clinical population. *Circulation*. 2011;124:2208–2214.

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

The Spectrum of Epidemiology Underlying Sudden Cardiac Death

Meiso Hayashi, Wataru Shimizu and Christine M. Albert

Circ Res. 2015;116:1887-1906
doi: 10.1161/CIRCRESAHA.116.304521
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571

ARTICLE IN PRESS

Journal of Arrhythmia ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Arrhythmia

journal homepage: www.elsevier.com/locate/joa

Review

Current topics in catecholaminergic polymorphic ventricular tachycardia

Naokata Sumitomo, MD, PhD*

Department of Pediatric Cardiology, Saitama Medical University International Medical Center, 1397-1 Yamane, Hidaka-City, Saitama 350-1298, Japan

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form
2 September 2015
Accepted 7 September 2015

Keywords:
Catecholaminergic polymorphic ventricular tachycardia (CPVT)
Ryanodine (*RyR2*)
Calsequestrine (*CASQ2*)
Delayed after depolarization
Left cardiac sympathetic denervation

ABSTRACT

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is induced by emotions or exercise in patients without organic heart disease and may be polymorphic or bidirectional in nature. The prognosis of CPVT is not good, and therefore prevention of sudden death is of utmost importance. Genetic variants of CPVT include *RyR2*, *CASQ2*, *CALM2*, *TRD*, and possibly *KCNJ2* and *ANK2* gene mutations. Hypotheses that suggest the causes of CPVT include weakened binding of FKBP12.6 and *RyR2*, a store overload-induced Ca^{2+} release (SOICR), unzipping of intramolecular domain interactions in *RyR2*, and molecular and functional abnormalities caused by mutations in the *CASQ2* gene. The incidence of an *RyR2* anomaly in CPVTs is about 35–79%, whereas anomalies in the *CASQ2* gene account for 3–5% CPVTs. The ping-pong theory, suggesting that reciprocating delayed after depolarization induces bigeminy of the right and left bundle branches, may explain the pathogenesis of bidirectional ventricular tachycardia. Flecainide, carvedilol, left sympathetic nerve denervation, and catheter ablation of the PVC may serve as new therapeutic strategies for CPVT while gene-therapy may be applied to some types of CPVT in the future. Although, not all sudden cardiac deaths in CPVT patients are currently preventable, new medical and interventional therapies may improve CPVT prognosis.

Contents

1. Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is induced by emotional stress or exercise in patients without

* Tel.: +81 429 84 4111x8625; fax: +81 429 84 4121.
E-mail address: sumitomo@saitama-med.ac.jp

http://dx.doi.org/10.1016/j.joa.2015.09.008
1880-4276/

Please cite this article as: Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia (2015), http://dx.doi.org/10.1016/j.joa.2015.09.008i

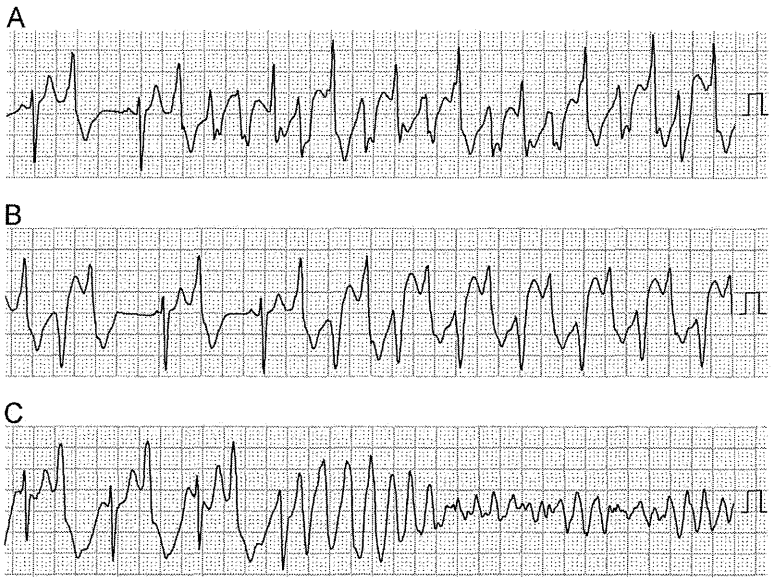

Fig. 1. Typical features of ventricular tachycardia in a patient with CPVT. (A) Polymorphic ventricular tachycardia. (B) Bidirectional ventricular tachycardia. (C) Rapid polymorphic ventricular tachycardia deteriorating into ventricular fibrillation. These electrocardiograms were recorded by Holter monitoring in the CM3 lead in the same patient.

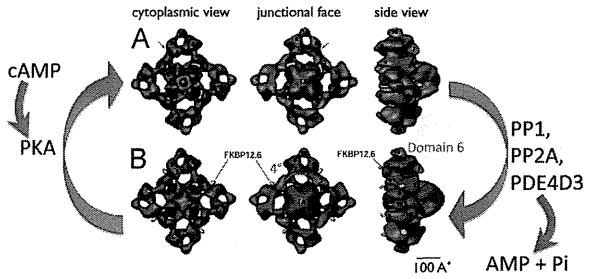

Fig. 2. Surface representations of *RyR2* 3D reconstructions with and without bound FKBP12.6. [7]. (A) High activity state of *RyR2*. A 3D map of *RyR2*, obtained by in vitro assembly of purified *RyR2* incubated with FKBP12.6 alone. (B) Low activity state of *RyR2*. A 3D map of *RyR2*, obtained by incubating *RyR2* with FKBP12.6 and an excess of FK506. FKBP12.6 is denoted by the blue dots. The major difference in these structures is observed in domain 6, which extends in the vertical direction (shown by the blue arrow), and the transmembrane assembly is rotated about 4° (shown by the blue arrow in the lower center panel). FKBP12.6: calstabin2, protein kinase A: PKA, phosphatase 1: PP1, phosphatase 2A: PP2A, phosphodiesterase 4D3: PDE 4D3, TA: transmembrane assembly.

organic heart disease and may be polymorphic or bidirectional (Fig. 1) [1–3]. This ventricular arrhythmia sometimes degenerates into rapid polymorphic ventricular tachycardia and ventricular fibrillation (Fig. 1) and may lead to syncope or sudden death. The incidence of CPVT is reported to be as high as 1:10,000, but its real prevalence is unclear.

2. Clinical manifestations and prognosis

The first clinical manifestations of CPVT are syncope or aborted sudden cardiac death during exercise or emotional stress and appear during the first or second decade of life [1–3]. CPVT differs from seizures, in that almost all syncopal events are associated with physical activity or emotional stress and do not occur during a resting state.

The prognosis of CPVT is very poor. About 40% patients die within 10 years of diagnosis [3]. Although prognosis in recent times could be better than previous reports, sudden death and severe brain damage are still reported in CPVT patients.

3. Diagnosis of CPVT

CPVT patients usually have a normal resting ECG, or just a lower heart rate than is normal for their age [3]. During exercise in these patients, monomorphic premature ventricular contractions (PVCs) increase, then polymorphic, or bidirectional PVC bigeminy appear, followed by bidirectional or polymorphic VT. Exercise induced supraventricular arrhythmias (atrial fibrillation, premature atrial contraction, and atrial tachycardia) are also common in the patients with CPVT [4]. The diagnostic criteria of CPVT are as follows [5]:

1. CPVT is diagnosed in the presence of a structurally normal heart, normal ECG, and unexplained exercise or catecholamine-induced bidirectional VT, polymorphic ventricular premature beats or VT in individuals < 40 years of age.
2. CPVT is diagnosed in patients (index case or family member) who have a pathogenic mutation.
3. CPVT is diagnosed in family members of a CPVT index case with a normal heart who manifests exercise-induced PVCs or bidirectional/polymorphic VT.
4. CPVT can be diagnosed in the presence of a structurally normal heart and coronary arteries, normal ECG, and unexplained exercise or catecholamine-induced bidirectional VT, polymorphic ventricular premature beats or VT in individuals > 40 years of age.

4. Mechanism of CPVT

The major pathogenic mechanism of CPVT is thought to involve the malfunction of *RyR2*. *RyR2* is a large tetrameric protein expressed on the sarcoplasmic reticulum (SR) membrane. *RyR2* is anchored to calsequestrin (CASQ2) by satellite proteins such as calmodulin (*CaM*), FKBP12.6, (calstabin2), protein kinase A (PKA), phosphatase 1 (PP1), and phosphatase 2A (PP2A) bound to the cytoplasmic region and junction, and triadin (TRD) bound to the luminal side [6].

Please cite this article as: Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia (2015), http://dx.doi.org/10.1016/j.joa.2015.09.008i

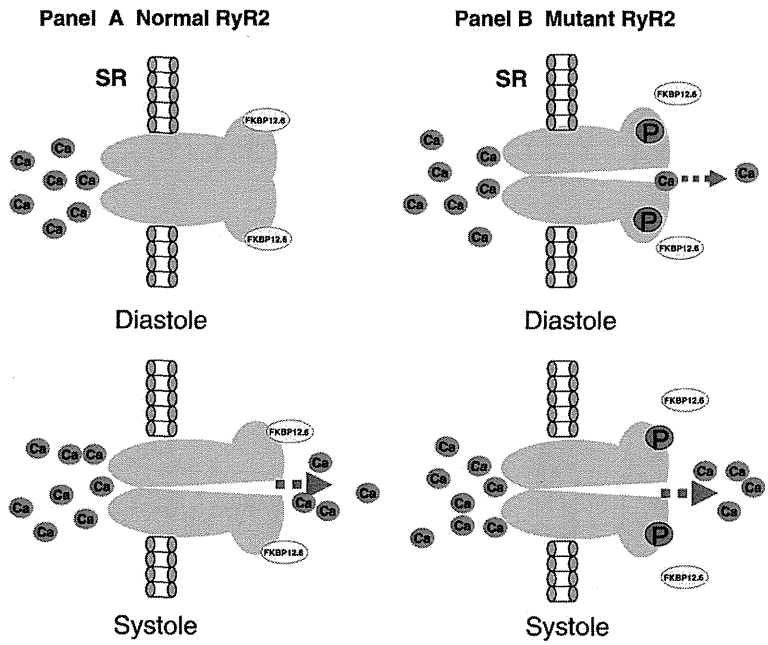

Fig. 3. FKBP12.6 dissociation from mutant *RyR2* in the pathogenesis of CPVT [8]. FKBP12.6 acts as a stabilizer that preserves the closed *RyR2* channel during diastole. Weakened binding affinity with FKBP12.6 may lead to a Ca^{2+} leak during diastole.

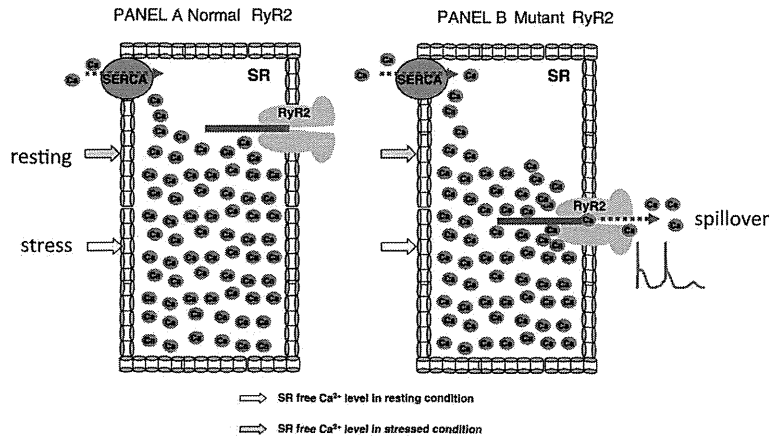

Fig. 4. The store overload-induced Ca^{2+} release (SOICR) hypothesis [8]. With normal *RyR2*, the resting and stress levels of free calcium are below the SOICR threshold (panel A). If the SOICR threshold falls below the level of free SR calcium as with mutant *RyR2*, a leak of Ca^{2+} will occur and generate a delayed after-depolarization.

A three-dimensional reconstruction of *RyR2* bound to FKBP12.6 is shown in Fig. 2B. When *RyR2* is bound to FKBP12.6, it forms a stable structure with closed pores, the domain 6 of *RyR2* was found to protrude into the luminal side, when observed from the junctional face after the transmembrane assembly (TA) was rotated counterclockwise by about 4° [7]. When unbound to FKBP12.6, *RyR2* assumes an open state (Fig. 2A) [7].

Several pathogenic hypotheses have been reported regarding the causes of CPVT [8]. The first theory suggests the dissociation of FKBP12.6 from *RyR2*. The normal *RyR2* channel is stabilized by FKBP12.6 and closes during diastole. With mutant *RyR2*, the binding affinity with FKBP12.6 is weakened, and phosphorylation of *RyR2* by protein kinase A (PKA) results in dissociation of FKBP12.6 from *RyR2*, resulting in open channels which may leak Ca^{2+} during diastole (Fig. 3).

The second hypothesis is a store overload-induced Ca^{2+} release (SOICR) theory [8]. With normal *RyR2*, the resting and stress levels of free Ca^{2+} are below the SOICR level. However, with mutant *RyR2*, the SOICR threshold drops below the level of free Ca^{2+} in the SR. This may cause a spillover of Ca^{2+} from the SR (Fig. 4).

The third hypothesis considers defective intramolecular domain interaction [8]. *RyR2* is stabilized by a tight zipping of the intramolecular structure. If a mutation interferes with this zipping structure, the intramolecular domain interaction is weakened, causing an unzipping of the interdomain structure and leads leaking of Ca^{2+} from the SR (Fig. 5).

Please cite this article as: Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia (2015), http://dx.doi.org/10.1016/j.joa.2015.09.008i

The fourth hypothesis suggests that the molecular and functional abnormalities are related to mutations in the *CASQ2* gene [8]. *CASQ2* is a Ca^{2+} storage protein inside the SR. The functional storage capacity of *CASQ2* or its reduced levels, may lead to increased levels of free Ca^{2+} inside the SR, leading to a Ca^{2+} leak during diastole (Fig. 6). It is also known that *CASQ2* stabilizes binding of *RyR2* with *TRD* and the junction.

This Ca^{2+} overload activates the forward mode of the Na^+/Ca^+ exchanger (NCX), increases the transient inward current (Iti), and induces ventricular arrhythmias due to delayed after depolarizations (DADs).

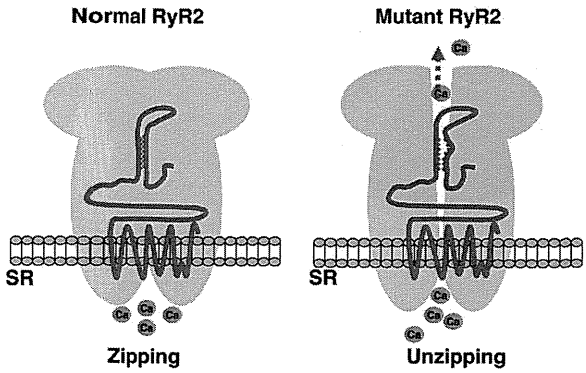

Fig. 5. Defective intramolecular domain interactions in *RyR2* mutations [8]. The N terminal domain and the central domain of *RyR2* interact with a tight "zipping" that serves to stabilize the channel (left panel). A mutation in either domain weakens this interaction (unzipping), which results in leaking of Ca^{2+} from *RyR2* (right panel).

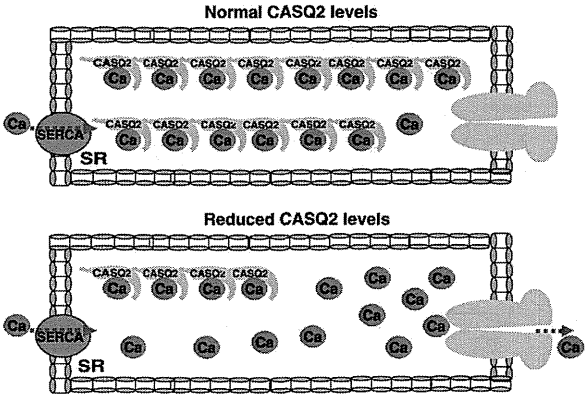

Fig. 6. Molecular and functional abnormalities related to mutations in the CASQ2 gene [8]. Storage of Ca^{2+} in the SR largely depends on the level and function of CASQ2 (upper panel). Decreased levels or function of CASQ2 results in increase in the free SR Ca^{2+} that may result in a Ca^{2+} leak from *RyR2* during diastole (lower panel).

5. Subtypes of CPVT

Several subtypes of CPVT have been reported (Table 1). The most common type of CPVT is caused by an anomaly in the *RyR2* gene (CPVT1) [9,10]. This accounts for more than 50% of CPVT cases. In our CPVT cohort, about 79% of the CPVT cases were related to an anomaly in the *RyR2* gene. The inheritance of CPVT1 is autonomic dominant, and sudden death was observed in about 10% of these patients. There were no sex differences noted in this CPVT.

The second most common type of CPVT is caused by a *CASQ2* gene anomaly (CPVT2) [11,12]. The inheritance of CPVT2 is autosomal recessive, and the rate of sudden death is higher than that observed in CPVT1. However, autosomal dominant mutations of *CASQ2* are also reported [13–15].

CPVT3 was reported in a family with showing a 7p22-p14 chromosome anomaly, but the gene responsible has not been identified yet [16]. Recently, calmodulin (*CALM*) [17] and triadin (*TRD*) [18] anomalies have been found to responsible for CPVT4 and CPVT5, respectively.

CALM is a protein that involves the calcium dependent I_{Ca} inactivation of the L-type Ca channel. Further, *CALM* also stabilizes the *RyR2* channel. Thus, a mutation in *CALM* may easily cause Ca^{2+} overload. *TRD* is a protein that connects *CASQ* to *RyR2*, and stabilizes the *RyR2* channel. A mutation in *TRD* may also result in a diastolic leak of Ca^{2+} and Ca^{2+} overload in the myocytes.

KCNJ2 encodes the cardiac inward rectifier K channel. A mutation in *KCNJ2* causes the Andersen–Tawil syndrome (LQT7), and is also reported in patients with exercise induced bi-directional VT [19]. Whether or not this type of mutation should be included as a subtype of CPVT is a matter of controversy. Mutations in the *ANK2* gene are well known as a cause of LQT4. Recently, a patient with an *ANK2* mutation was reported to have bi-directional VT [20]. This may be another disease related to CPVT.

A type of adult CPVT has also been reported [21,22]. In this disease, the patients are predominantly female, with CPVT onset at the age of around 40 years, and no sudden death is reported. We believe that this may not be a specific type of CPVT, but rather a mild form of the disease.

In the Japanese CPVT registry, 78 patients (M:F=26:52, age= 11.2 ± 8.2 years) were enrolled. In this registry, only 6% of the cases were familial cases whereas 94% of the cases were sporadic

Table 1
Subtypes of CPVT.

Subtypes	Juvenile type							Adult type
	CPVT1	CPVT2	CPVT3	CPVT4	CPVT5	CPVT related diseases		
						ATS	LQT4	
Incidence (%)	50–60	1	≪1	≪1	≪1	≪1	≪1	≈30
Inheritance	AD	AR	AR	AD	Sporadic	AD	AD	Sporadic
Onset of symptoms	10 years	7 years	10 years	4 years	2, 26 years	14, 9, 17 years	?	> 20 years (40 years)
Sex	M:F=1:1	M:F=1:1	M:F=1:1	M:F=1:1	M=3	F > M?	?	F ≫ M
Chromosome locus	1q43	1p13.1	7p22–p14	14q32.11	6q22.31	17q24.3	4q25-26	
Gene	*RyR2*	*CASQ2*	?	*CALM1*	*TRD*	*KCNJ2*	*ANK2*	*RyR2* ≈ 30%
Protein				CaM		$K_{ir}2.1\alpha$	Ankyrin-B	
Sudden death (%)	≈10	≈42	≈75	≈18	≈25	?	?	0

Please cite this article as: Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia (2015), http://dx.doi.org/10.1016/j.joa.2015.09.008i

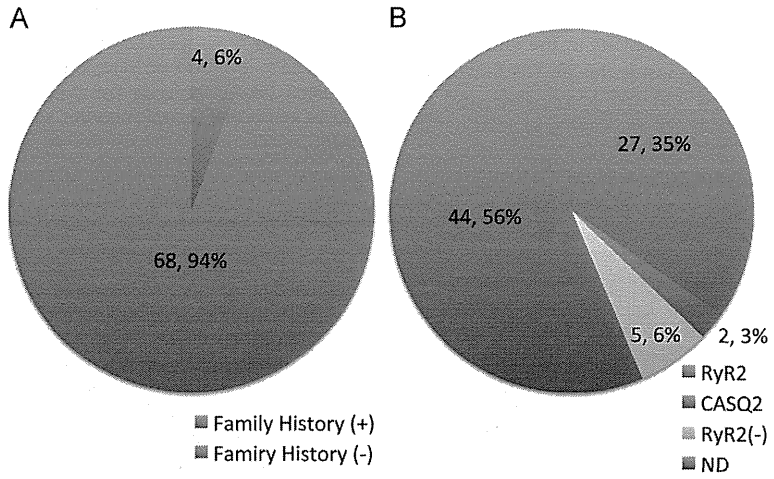

Fig. 7. Family history and gene anomalies in the Japanese registry. (A) Family history in the registry. (B) Gene mutations ND; gene testing was not performed.

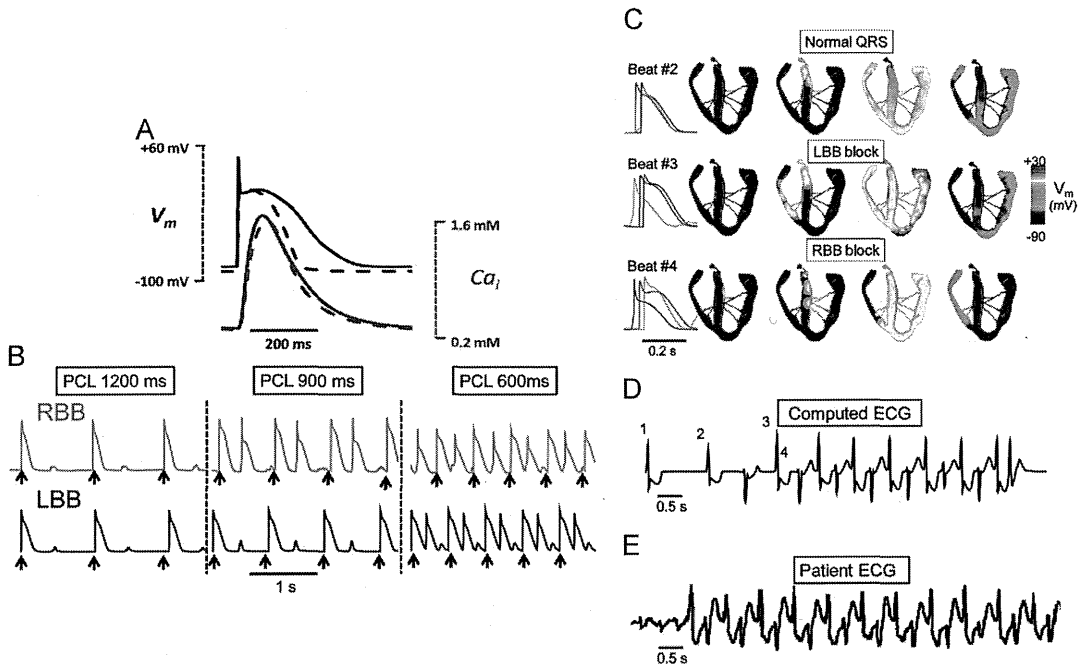

Fig. 8. A possible mechanism for bidirectional ventricular tachycardia: ping-pong in the His–Purkinje system [27]. (A) Comparison of simulated rabbit ventricular (dashed line) and Purkinje (solid line) action potentials (APs) and Cai transients during pacing at 600 ms. (B) Rate dependence of delayed after depolarizations (DADs) and bigeminy in Purkinje cell AP models. For the green trace, the rate threshold for DAD-induced bigeminy was 67 bpm (pacing cycle length [PCL] 900 ms), such that pacing (black arrows) at both 900 and 600 ms induced bigeminy. For the purple trace, the bigeminy rate threshold was 100 bpm (PCL 600 ms), such that pacing at 600 ms, but not 900 ms, induced bigeminy. LBB: left bundle branch; RBB: right bundle branch. (C) Voltage snapshots depicting the activation sequence at BVT onset Beat #2 is the last paced beat, with normal activation. Beat #3 is the first beat of BVT, due to a DAD-triggered action potential (AP) arising in the right bundle branch (RBB), resulting in QRS with a left bundle branch (LBB) block pattern. Beat #4 is the second beat of BVT, due to a DAD-triggered AP arising in the LBB and results in a QRS with RBB block pattern. Traces on the right show the timing of APs recorded from the His bundle (red), RBB (green), and LBB (purple). (D) Computed ECG from the simulation in A, showing BVT. (E) ECG recorded in a patient during BVT.

(Fig. 7A). In this cohort, 56% of the patients had not undergone genetic testing. However, of the 46% patients who underwent genetic testing, 79% of the patients had an *RyR2* gene anomaly, 6% had a *CASQ2* gene anomaly, and in 15% of the patients the specific causative gene anomaly was unknown (Fig. 7B). The estimated *RyR2* genotype percentage is reported to range from 35% [23] up to 65% [24,25], and the *CASQ2* genotyped patients are estimated to account for approximately 3–5% [25].

The proportions of familial cases reported in other studies were 21.3% [26] and 30% [21]. The lower percentage of familial cases observed in our cohort may be because half of the registered cases are over 15 years old, at which time only information of familial

Please cite this article as: Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia (2015), http://dx.doi.org/10.1016/j.joa.2015.09.008i

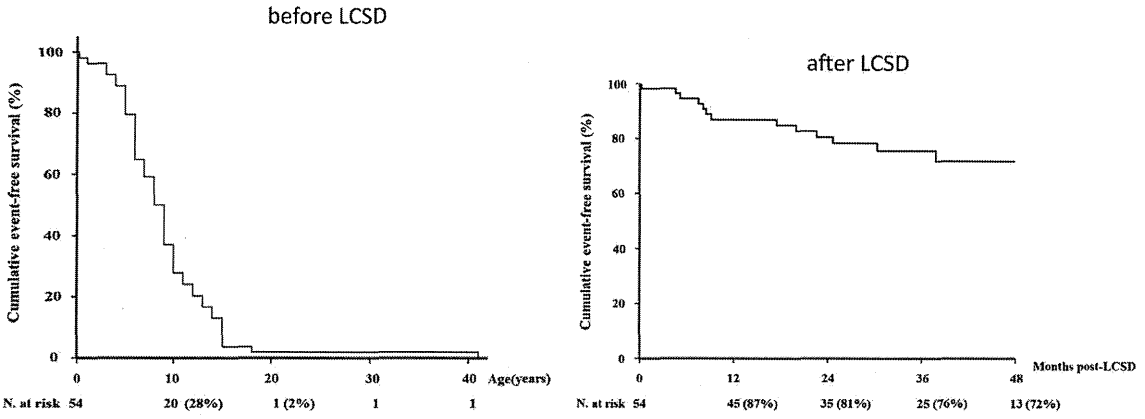

Fig. 9. Kaplan–Meier curve of cumulative survival to a first major cardiac event before and after left cardiac sympathetic denervation (LCSD) in symptomatic patients with CPVT [38]. In 63 patients with CPVT, the cumulated event free survival significantly improved after LCSD.

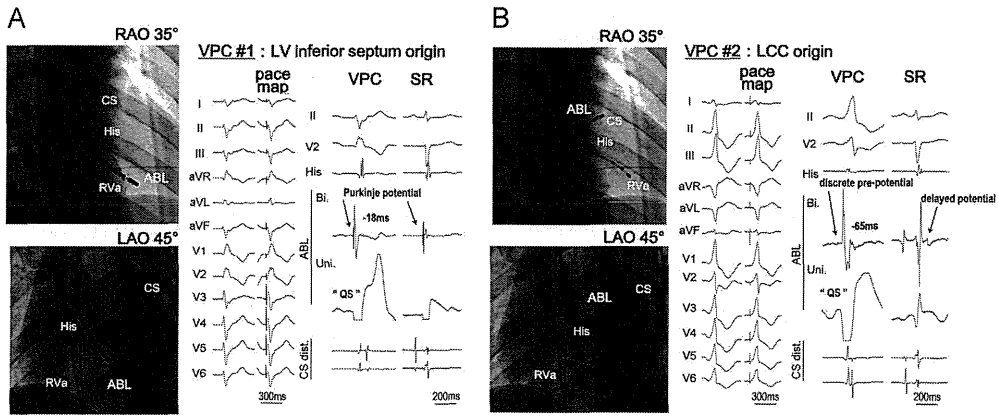

Fig. 10. Pace mapping of PVC in a patient with CPVT [43]. (A) A perfect pace map of the second beat of the CPVT was obtained on the left ventricular septum. Purkinje potential at that point was recorded during the PVC and sinus rhythm. (B) A perfect pace map of the first beat of a PVC was obtained at the left coronary cusp. A discrete pre-potential was recorded during the PVC, and a delayed potential was recorded during sinus rhythm.

history was taken without exercise or genetic testing. This may result in an apparently lower percentage of familial cases. Kawamura et al. have reported that *RyR2* positive CPVT cases are more likely to have clinically diagnosed CPVT-affected family members with bidirectional VT, and sinus bradycardia [26].

6. The mechanism of bidirectional VT

Bidirectional VT is the most characteristic feature of CPVT. In the His–Purkinje system, DAD induced bigeminy may differ depending on whether they are induced by the right bundle branch or the left bundle branch. The right bundle branch (RBB) caused a DAD induced bigeminy at a pacing rate of 900 ms (Fig. 8B), whereas the left bundle branch (LBB) induced a bigeminy at a pacing rate of 600 ms (Fig. 8B) [27]. In these situations, the sinus rate exceeded the threshold of the RBB-DAD induced bigeminy rate, and the beat after the sinus beat may have been induced from the RBB, resulting in a LBB block (LBBB) type PVC. The coupling interval of the normal sinus beat to the LBBB type PVC exceeded the threshold of the LBB-DAD induced bigeminy, and the next beat arose from LBB, resulting in a RBB block (RBBB) type PVC. When the coupling interval of the LBBB type PVC and RBBB type PVC exceeded the threshold of the RBBDAD induced bigeminy, the next beat arose from the RBB followed by a beat from the LBB, one after the other (Fig. 8C) [27]. This computer simulation suggests a mechanism for the bidirectional VT.

7. Therapy for the CPVT

7.1. β Blockers

The long acting β blocker, nadolol, is preferred for prophylactic treatment of CPVT. Propranolol is also an effective medication. However, β blockers cannot completely suppress the arrhythmic events in CPVT patients [28].

Carvedilol is reported to inhibit the SOICR in an HEK 293 cell culture model. Among various β blockers, only carvedilol inhibits *RyR2* activity [29]. Thus, carvedilol may be an effective β blocker for CPVT, but its β blocking effect may be weak in comparison to the other β blockers. Therefore, the efficacy of carvedilol needs to be further investigated.

7.2. Verapamil

Verapamil has also shown beneficial effects in some CPVT patients [30,31]. However, the long-term efficacy of verapamil is still controversial.

Please cite this article as: Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia (2015), http://dx.doi.org/10.1016/j.joa.2015.09.008i

7.3. Flecainide

Flecainide is an effective medication for CPVT [32–34]. Flecainide treatment shows improvement of ventricular arrhythmias in 74% of the genotype positive CPVT cases [32], and in 92% of the genotype negative CPVT cases [34]. Flecainide is thought to function by direct suppression of the *RyR2* receptor. Among the Class I anti-arrhythmic medications, only flecainide and propafenone inhibit *RyR2* activity [35]. However, recent report denies the direct suppression of *RyR2* by flecainide [36]. That may suggest another mechanism of flecainide, such as inhibition of NCX.

7.4. Left cardiac sympathetic denervation

Left cardiac sympathetic denervation is reported to be a useful therapeutic method for suppressing ventricular arrhythmias in CPVT patients [37,38]. In patients with uncontrollable ventricular arrhythmias, left cardiac sympathetic denervation is highly useful in controlling ventricular tachyarrhythmias (Fig. 9). The rate of complications involving Horner syndrome is very low if denervation is performed in the lower half of the T1 sympathetic ganglion through the T4 ganglion [38].

7.5. ICD

Implantation of an ICD should be considered in patients in the absence of controlled optimal therapy [39]. However, implantation of an ICD in children still has a number of technical problems [40]. Moreover, inappropriate or painful shocks may increase the risk of further ventricular arrhythmias, and electrical storms that may result in lethal events.

7.6. Catheter ablation

Pulmonary vein isolation is reported to be effective in some CPVT patients with atrial fibrillation [41]. Purkinje cells are reported to be more arrhythmogenic than ventricular myocytes in a mutant knockout mouse model of CPVT [42]. The onset of CPVT may be initiated from Purkinje cells. Successful catheter ablation has been reported at the site of Purkinje potentials or discrete prepotentials (Fig. 10) [43].

7.7. Gene therapy

The homozygous R33Q knock-in mouse has a dysfunctional *CASQ2*, which may cause CPVT. In this mouse model, isoproterenol induced DADs, which were markedly reduced after 12 months following infection with an adenoviral vector (serotype 9), that carried the normal *CASQ2* gene [44]. This report suggested the possible use of gene therapy for some types of CPVT in the future.

Conflict of interest

All authors declare no conflict of interest related to this study.

Acknowledgment

This work was supported by Health Science Research grant from the Ministry of Health, Labour and Welfare of Japan for Clinical Research on Measures for Intractable Diseases (2016-032).

References

[1] Coumel P, Fidelle J, Lucet V, et al. Catecholamine-induced severe ventricular arrhythmias with Adams–Stokes syndrome in children: report of four cases. Br Heart J 1978;40(Suppl.):S28–37.
[2] Leenhardt A, Lucet V, Denjoy I, et al. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. Circulation 1995;91:1512–9.
[3] Sumitomo N, Harada K, Nagashima M, et al. Catecholaminergic polymorphic ventricular tachycardia: electrocardiographic characteristics and optimal therapeutic strategies to prevent sudden death. Heart 2003;89:66–70.
[4] Sumitomo N, Sakurada H, Taniguchi K, et al. Association of atrial arrhythmia and sinus node dysfunction in patients with catecholaminergic polymorphic ventricular tachycardia. Circ J 2007;71:1551–4.
[5] Priori SG, Wilde AA, Horie M, et al. Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. J Arrhythm 2014;30:29–47.
[6] Yano M, Yamamoto T, Kobayashi S, et al. Role of ryanodine receptor as a $Ca^2(+)$ regulatory center in normal and failing hearts. J Cardiol 2009;53:1–7.
[7] Sharma MR, Jeyakumar LH, Fleischer S, et al. Three-dimensional visualization of FKBP12.6 binding to an open conformation of cardiac ryanodine receptor. Biophys J 2006;90:164–72.
[8] Liu N, Rizzi N, Boveri L, et al. Ryanodine receptor and calsequestrin in arrhythmogenesis: what we have learnt from genetic diseases and transgenic mice. J Mol Cell Cardiol 2009;46:149–59.
[9] Priori SG, Napolitano C, Tiso N, et al. Mutations in the cardiac ryanodine receptor gene (h*RyR2*) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation 2001;103:196–200.
[10] Laitinen PJ, Brown KM, Piippo K, et al. Mutations of the cardiac ryanodine receptor (*RyR2*) gene in familial polymorphic ventricular tachycardia. Circulation 2001;103:485–90.
[11] Lahat H, Eldar M, Levy-Nissenbaum E, et al. Autosomal recessive catecholamine- or exercise-induced polymorphic ventricular tachycardia: clinical features and assignment of the disease gene to chromosome. Circulation 2001;103:2822–7.
[12] Lahat H, Pras E, Olender T, et al. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. Am J Hum Genet 2001;69:1378–84.
[13] de la Fuente S, Van Langen IM, Postma AV, et al. A case of catecholaminergic polymorphic ventricular tachycardia caused by two calsequestrin 2 mutations. Pacing Clin Electrophysiol 2008;31:916–9.
[14] Postma AV, Denjoy I, Hoorntje TM, et al. Absence of calsequestrin2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. Circ Res 2002;91:e21–6.
[15] Roux-Buisson N, Egea G, Denjoy I, et al. Germline and somatic mosaicism for a mutation of the ryanodine receptor type2 gene: implication for genetic counseling and patient caring. Europace 2011;13:130–2.
[16] Bhuiyan ZA, Hamdan MA, Shamsi ET, et al. A novel early onset lethal form of catecholaminergic polymorphic ventricular tachycardia maps to chromosome. J Cardiovasc Electrophysiol 2007;18:1060–6.
[17] Nyegaard M, Overgaard MT, Sondergaard MT, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. Am J Hum Genet 2012;91:703–12.
[18] Roux-Buisson N, Cacheux M, Fourest-Lieuvin A, et al. Absence of triadin, a protein of the calcium release complex, is responsible for cardiac arrhythmia with sudden death in human. Hum Mol Genet 2012;21:2759–67.
[19] Vega AL, Tester DJ, Ackerman MJ, et al. Protein kinase A-dependent biophysical phenotype for V227F-KCNJ2 mutation in catecholaminergic polymorphic ventricular tachycardia. Circ Arrhythm Electrophysiol 2009;2:540–7.
[20] Mohler PJ, Splawski I, Napolitano C, et al. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. Proc Natl Acad Sci USA 2004;101:9137–42.
[21] Sy RW, Gollob MH, Klein GJ, et al. Arrhythmia characterization and long-term outcomes in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 2011;8:864–71.
[22] Sumitomo N. Are there juvenile and adult types in patients with catecholaminergic polymorphic ventricular tachycardia? Heart Rhythm 2011;8:872–3.
[23] Bai R, Napolitano C, Bloise R, et al. Yield of genetic screening in inherited cardiac channelopathies: how to prioritize access to genetic testing. Circ Arrhythm Electrophysiol 2009;2:6–15.
[24] Priori SG, Napolitano C, Memmi M, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation 2002;106:69–74.
[25] Ackerman MJ, Priori SG, Willems S, et al. HRS/HER a expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm 2011;8:1308–39.
[26] Kawamura M, Ohno S, Naiki N, et al. Genetic background of catecholaminergic polymorphic ventricular tachycardia in Japan. Circ J 2013;77:1705–13.
[27] Baher AA, Uy M, Xie F, et al. Bidirectional ventricular tachycardia: ping pong in the His–Purkinje system. Heart Rhythm 2011;8:599–605.
[28] Hayashi M, Denjoy I, Extramiana F, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. Circulation 2009;119:2426–34.

Please cite this article as: Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia (2015), http://dx.doi.org/10.1016/j.joa.2015.09.008i

[29] Zhou Q, Xiao J, Jiang D, et al. Carvedilol and its new analogs suppress arrhythmogenic store overload-induced Ca^{2+} release. Nat Med 2011;17:1003–9.
[30] Rosso R, Kalman JM, Rogowski O, et al. Calcium channel blockers and beta-blockers versus beta-blockers alone for preventing exercise-induced arrhythmias in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 2007;4:1149–54.
[31] Swan H, Laitinen P, Kontula K, et al. Calcium channel antagonism reduces exercise-induced ventricular arrhythmias in catecholaminergic polymorphic ventricular tachycardia patients with *RyR2* mutations. J Cardiovasc Electrophysiol 2005;16:162–6.
[32] Watanabe H, Chopra N, Laver D, et al. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. Nat Med 2009;15:380–3.
[33] van der Werf C, Kannankeril PJ, Sacher F, et al. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. J Am Coll Cardiol 2011;57:2244–54.
[34] Watanabe H, van der Werf C, Roses-Noguer F, et al. Effects of flecainide on exercise-induced ventricular arrhythmias and recurrences in genotype-negative patients with catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 2013;10:542–7.
[35] Hwang HS, Hasdemir C, Laver D, et al. Inhibition of cardiac Ca^{2+} release channels (*RyR2*) determines efficacy of class I antiarrhythmic drugs in catecholaminergic polymorphic ventricular tachycardia. Circ Arrhythm Electrophysiol 2011;4:128–35.
[36] Bannister ML, Thomas NL, Sikkel MB, et al. The mechanism of flecainide action in CPVT does not involve a direct effect on RyR2. Circ Res 2015;116:1324–35.
[37] Wilde AA, Bhuiyan ZA, Crotti L, et al. Left cardiac sympathetic denervation for catecholaminergic polymorphic ventricular tachycardia. N Engl J Med 2008;358:2024–9.
[38] De Ferrari GM, Dusi V, Spazzolini C, et al. Clinical management of catecholaminergic polymorphic ventricular tachycardia: the role of left cardiac sympathetic denervation. Circulation 2015;131:2185–93.
[39] van der Werf C, Zwinderman AH, Wilde AA. Therapeutic approach for patients with catecholaminergic polymorphic ventricular tachycardia: state of the art and future developments. Europace 2012;14:175–83.
[40] Sumitomo N. Device therapy in children and patients with congenital heart disease. J Arrhythmia 2014;30:428–32.
[41] Sumitomo N, Nakamura T, Fukuhara J, et al. Clinical effectiveness of pulmonary vein isolation for arrhythmic events in a patient with catecholaminergic polymorphic ventricular tachycardia. Heart Vessels 2010;25:448–52.
[42] Kang G, Giovannone SF, Liu N, et al. Purkinje cells from *RyR2* mutant mice are highly arrhythmogenic but responsive to targeted therapy. Circ Res 2010;107:512–9.
[43] Kaneshiro T, Naruse Y, Nogami A, et al. Successful catheter ablation of bidirectional ventricular premature contractions triggering ventricular fibrillation in catecholaminergic polymorphic ventricular tachycardia with *RyR2* mutation. Circ Arrhythm Electrophysiol 2012;5:e14–7.
[44] Denegri M, Bongianino R, Lodola F, et al. Single delivery of an adeno-associated viral construct to transfer the CASQ2 gene to knock-in mice affected by catecholaminergic polymorphic ventricular tachycardia is able to cure the disease from birth to advanced age. Circulation 2014;129:2673–81.

Please cite this article as: Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia (2015), http://dx.doi.org/10.1016/j.joa.2015.09.008i

International Journal of Cardiology 207 (2016) 349–358

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Targeted resequencing identifies *TRPM4* as a major gene predisposing to progressive familial heart block type I

Xavier Daumy [a,b,c,1], Mohamed-Yassine Amarouch [d,1,2], Pierre Lindenbaum [a,b,c,e], Stéphanie Bonnaud [a,b,c,e], Eric Charpentier [a,b,c], Beatrice Bianchi [d], Sabine Nafzger [d], Estelle Baron [a,b,c], Swanny Fouchard [d], Aurélie Thollet [d], Florence Kyndt [a,b,c,e], Julien Barc [a,b,c], Solena Le Scouarnec [a,b,c], Naomasa Makita [f], Hervé Le Marec [a,b,c,e], Christian Dina [a,b,c,e], Jean-Baptiste Gourraud [a,b,c,e], Vincent Probst [a,b,c,e], Hugues Abriel [d,*,1], Richard Redon [a,b,c,e,1], Jean-Jacques Schott [a,b,c,e,**,1]

[a] *Institut National de la Santé et de la Recherche Médicale (INSERM) Unité Mixte de Recherche (UMR) 1087, l'institut du thorax, Nantes, France*
[b] *Centre National de la Recherche Scientifique (CNRS) UMR 6291, l'institut du thorax, Nantes, France*
[c] *Université de Nantes, l'institut du thorax, Nantes, France*
[d] *Department of Clinical Research, and Swiss National Centre of Competence in Research (NCCR) TransCure, University of Bern, Switzerland*
[e] *Centre Hospitalier Universitaire (CHU) de Nantes, l'institut du thorax, Service de Cardiologie, Nantes, France*
[f] *Molecular Physiology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan*

ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form 5 November 2015
Accepted 1 January 2016
Available online 11 January 2016

Keywords:
TRPM4
Atrio-ventricular block
PFHBI
Gain-of-function mutation

ABSTRACT

Background: Progressive cardiac conduction disease (PCCD) is one of the most common cardiac conduction disturbances. It has been causally related to rare mutations in several genes including *SCN5A*, *SCN1B*, *TRPM4*, *LMNA* and *GJA5*.
Methods and results: In this study, by applying targeted next-generation sequencing (NGS) in 95 unrelated patients with PCCD, we have identified 13 rare variants in the *TRPM4* gene, two of which are currently absent from public databases. This gene encodes a cardiac calcium-activated cationic channel which precise role and importance in cardiac conduction and disease is still debated. One novel variant, *TRPM4*-p.I376T, is carried by the proband of a large French 4-generation pedigree. Systematic familial screening showed that a total of 13 family members carry the mutation, including 10 out of the 11 tested affected individuals versus only 1 out of the 21 unaffected ones. Functional and biochemical analyses were performed using HEK293 cells, in whole-cell patch-clamp configuration and Western blotting. *TRPM4*-p.I376T results in an increased current density concomitant to an augmented TRPM4 channel expression at the cell surface.
Conclusions: This study is the first extensive NGS-based screening of *TRPM4* coding variants in patients with PCCD. It reports the third largest pedigree diagnosed with isolated Progressive Familial Heart Block type I and confirms that this subtype of PCCD is caused by mutation-induced gain-of-expression and function of the TRPM4 ion channel.

1. Introduction

Progressive cardiac conduction defect (PCCD) was first described in the sixties by Lenègre [1] and Lev [2] as a fibrosis process affecting the conduction system. It is one of the most common cardiac conduction disturbances characterized by a progressive alteration of cardiac conduction through the His-Purkinje system with right or left bundle branch block (RBBB or LBBB) and widening of QRS complexes, leading to complete atrioventricular block (AVB), syncope and sudden death.

Familial cases of PCCD have been reported with an autosomal dominant inheritance and causally related to rare mutations in genes involved in cardiac impulse propagation (*SCN5A* [3,4], *SCN1B* [5] and *TRPM4* [6,7]), in the structure of the nuclear lamina (*LMNA* [8,9]) and in cell-to-cell communication (*GJA5* [10]).

Among these genes, *TRPM4*, encoding a calcium-activated cationic channel that is expressed in Purkinje fibers and nodal tissue [6,7], has first been linked to progressive familial heart block type I (PFHBI) in two large pedigrees, respectively from South Africa [6] and Lebanon

* Correspondence to: H. Abriel, University of Bern, Department of Clinical Research, Murtenstrasse, 35, 3010 Bern, Switzerland.
** Correspondence to: J.-J. Schott, Inserm UMR 1087, l'institut du thorax IRT-UN, 8 quai Moncousu, 44007 Nantes, France.
E-mail address: Hugues.Abriel@dkf.unibe.ch (H. Abriel), jjschott@univ-nantes.fr (J.-J. Schott).
[1] These authors contributed equally to this work.
[2] Current affiliation: Materials, Natural Substances, Environment & Modeling Laboratory, University of Sidi Mohamed Ben Abdellah- Fes, Multidisciplinary Faculty of Taza, Taza, Morocco.

http://dx.doi.org/10.1016/j.ijcard.2016.01.052
0167-5273/

[7]. PFHBI is associated with a progressive impairment of the His bundle branches conduction, typically starting with RBBB and then left anterior hemiblock (LAHB) and that may progress to a complete AVB. QRS duration increases with time while PR and QTc intervals remain constant [11–13].

Conduction defects in TRPM4-dependent familial cases were shown to be related to gain-of-function mutations proposed to be caused by a reduction of the deSUMOylation of TRPM4 channels and an impaired endocytosis resulting in stabilization and overexpression of mutant channels at the plasma membrane [6,7]. Since these two seminal reports, eighteen gain- or loss-of-function variants have been identified as causing diverse forms of cardiac conduction defects and/or Brugada syndrome [14–16].

Here, using next generation sequencing (NGS) technologies, we report novel *TRPM4* variants including one (c.T1127C; p.I376T) segregating with the third largest reported PCCD family. This missense mutation segregates in 39 relatives of a 4-generation pedigree and was observed to lead to gain-of-expression and function of the mutant channel. These findings strongly support a central role of *TRPM4* in cardiac conduction and cardiac conduction disorders.

2. Methods

2.1. Patient phenotyping

The study was conducted according to the French guidelines for genetic research and approved by the ethic committee of the Nantes University Hospital. A written consent was obtained for each family member who accepted to participate in the study.

The investigation included a physical examination with particular attention to the cardiovascular system and a 12-lead ECG. Heart rate, PR interval, QRS, QTc duration, P and QRS axes were measured automatically at rest (Mac Vu Marquette Inc., Milwaukee, Wisconsin, USA). Conduction defects were defined using the conventional classification [17, 18]. Two expert physicians, blinded to the clinical status, independently and systematically reviewed the ECG parameters.

Because of the prevalence of minor conduction defects in the general population and in order to decrease the risk of misclassification, only the most obviously affected patients were considered as affected. QRS axis was classified as normal when its value was between −30° and +90°. PR duration shorter than 210 ms was considered as normal. Patients were considered as affected if they have been implanted with a pacemaker (PM) for PCCD or if they have an ECG showing a major conduction defect (complete AVB, complete RBBB, complete LBBB, parietal block (PB) defined as a QRS wider than 115 ms without morphology of RBBB or LBBB, LAHB or left posterior hemiblock (LPHB)). Given the progressive nature of the disease, only patients older than 45 without any conduction defect were considered as unaffected. All the other patients were considered as undetermined and not included for the evaluation of the ECG parameters. Cardiac morphological diseases were excluded by echocardiography in all patients.

2.2. Targeted sequencing

Genomic DNA was extracted from peripheral blood lymphocytes by standard protocols. The DNA yields were assessed by measurements using Quant-IT™ dsDNA Assay Kit, Broad Range (Life Technologies, Q33130). The purity of the DNA was assessed by spectrophotometry (OD 260:280 and 260:230 ratios) using a Nanodrop instrument (Thermo Scientific). DNA integrity was assessed by separation in a E-Gel® 96 Agarose Gels, 1% (Life Technologies, G700801). For multiplex amplification, we used the HaloPlex™ Target Enrichment System (Agilent Technologies, 1–500 kb, ILMFST, 96 reactions, G9901B), Protocol Version D.2 (November, 2012). We applied a custom HaloPlex™ design enabling high-throughput sequencing of the coding regions of 45 genes previously linked to cardiac arrhythmias or conduction defects and/or sudden cardiac death, including 19 genes known or suspected to be involved in PCCD. In this study we focused solely on the relevant 19 PCCD genes including *SCN5A*, *SCN1B*, *TRPM4*, *GJA5* and *LMNA* that have already been associated with isolated cardiac conduction defects together with *GJA1*, *GJC1*, *SCN10A*, *NKX2-5*, *TBX5*, *SNTA1*, *PRKAG2*, *RYR2*, *EMD*, *BMP2*, *BMPR1A*, *GATA4*, *MSX2* and *TNNI3K* as likely candidate genes. The targeted coding regions (exons) ± 10 bp correspond to 141 kb of genomic sequence.

Target enrichment and sequencing were performed as previously described [19]. First, 200 ng of gDNA samples were digested in eight different restriction reactions, each containing two restriction enzymes, to create a library of gDNA restriction fragments. These gDNA restriction fragments were hybridized to the HaloPlex probe capture library. Probes are designed to hybridize and circularize targeted DNA fragments. During the hybridization process, Illumina sequencing motifs including index sequences were incorporated into the targeted fragments. The circularized target DNA biotinylated HaloPlex probe complexes were captured on magnetic streptavidin beads. We proceeded to a ligation reaction of the circularized complexes followed by an elution reaction before PCR amplification. The amplified target DNA was purified using AMPure XP bead (Beckman Coulter, A63881). To validate enrichment of target DNA in each library sample by microfluidics analysis, we used the 2200 TapeStation (Agilent Technologies, G2964AA), with D1K ScreenTape (Agilent Technologies, 5067–5361), and D1K Reagents (Agilent Technologies, 5067–5362). We ensured that the majority of amplicons range from 175 to 625 bp. Finally we quantified each library by qPCR using KAPA Library Quantification Kit (Clinisciences, KK4854). Libraries were pooled to an equimolar concentration and DNA was then denatured with NaOH. Finally libraries pool was diluted to a 4 pM final concentration before proceeding to 100 bp paired-end Illumina sequencing on HiSeq.

2.3. Detection of rare coding variation in TRPM4

Raw sequence reads were aligned to the human reference genome (GRCh37) using BWAMEM (version 0.7.5a) after removing sequences corresponding to Illumina adapters with Cutadapt v1.2. GATK was used for indel realignment and base recalibration, following GATK DNAseq Best Practices. Variants were called for each sample separately using GATK UnifiedGenotyper (version 2.8) and Samtools mpileup (version 0.1.19), and variants were considered for further analyses if found by both GATK and Samtools with a minimum quality score of 25.

Variants were considered of interest if: 1—They present a potential pathogenicity as predicted by Variant Effect Predictor (Ensembl). Variants were considered as having a potential functional consequence if they were annotated with one or more of the following SO terms for at least one RefSeq transcript: "transcript_ablation" (SO:0001893), "splice_donor_variant" (SO:0001575), "splice_acceptor_variant" (SO:0001574), "stop_gained" (SO:0001587), "frameshift_variant" (SO:0001589), "stop_lost" (SO:0001578), "initiator_codon_variant" (SO:0001582), "inframe_insertion" (SO:0001821), "inframe_deletion" (SO:0001822), "missense_variant" (SO:0001583), "transcript_amplification" (SO:0001889); 2—They were rare that is if the minor allele frequency (MAF) was <1% compared to the 1000 genomes phase 1 data (379 individuals of European origin, integrated release v3, downloaded from ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20110521), to the NHLBI GO Exome Sequencing Project (ESP) data – Exome Variant Server (EVS) (4300 individuals of European origin, ESP6500SI-V2 release, downloaded from http://evs.gs.washington.edu/EVS), and to the Exome Aggregation Consortium (ExAC) data (60,706 unrelated individuals including more than 33,300 non-Finnish European individuals, release v1, downloaded from ftp://ftp.broadinstitute.org/pub/ExAC_release).

In case of missense variants SIFT [20] and PolyPhen-2 (PPH-2) [21] were used to predict the impact of the amino acid substitutions. Filtering was performed using Knime4Bio [22].

2.4. Segregation analysis

Familial segregation analyses were carried out by bidirectional direct sequencing of amplified genomic DNA amplicons with variant-specific primers (forward: CCTCCATCCCTTTGGACAG; reverse: CAGGCCAGGA AAGGTGTCTA) using the "Big Dye Terminator v3.1 Cycle Sequencing Kit" (Applied Biosystems - Life Technologies) following the manufacturer's recommendations. The capillary sequencing was performed on Applied Biosystems 3730 DNA Analyzer using standard procedures provided by Applied Biosystems (Life Technologies).

The RefSeq NM_017636.3 transcript has been used to compare our sequencing data.

Linkage was assessed between the variant I376K and the disease using standard method comparing likelihood under a recombination fraction of 50% (no linkage) and 0% (full linkage). LOD score [23] calculation was performed with Superlink-Online SNP version 1.1 (http://cbl-hap.cs.technion.ac.il/superlink-snp/main.php) [24].

We postulated a rare causal variant (frequency set at 1/10,000) and dominant model with high penetrance (80%). The prevalence is 5% and therefore, the phenocopy rate is 0.0499.

A LOD score higher than 3 is considered as significant for linkage.

2.5. Cell culture and transfection

Human embryonic kidney (HEK293) cells were cultured with DMEM medium supplemented with 4 mM Glutamine, 10% FBS and a cocktail of streptomycin–penicillin antibiotics. For the electrophysiological studies, the cells were transiently transfected with 80 ng of HA-TRPM4 WT or HA-TRPM4 p.I376T in a 35 mm dish mixed with 4 μl of JetPEI (Polyplus transfection, Illkirch, France) and 46 μl of 150 mM NaCl. The cells were incubated for 24 h at 37 °C with 5% CO2. All transfections included 400 ng of cDNA encoding CD8 antigen as a reporter gene. Anti-CD8 beads (Dynal®, Oslo, Norway) were used to identify transfected cells, and only CD8-displaying cells were analyzed. Cells were used 24 h after transfection.

For the biochemical studies, HEK 293-cells were transiently transfected with 960 ng of either HA-TRPM4 WT, HA-TRPM4 p.I376T variants or empty vector (pcDNA4TO) in a P100 dish (BD Falcon, Durham, North Carolina, USA) mixed with 30 μl of JetPEI (Polyplus transfection, Illkirch, France) and 250 μl of 150 mM NaCl. The cells were incubated for 48 h at 37 °C with 5% CO2.

2.6. Cell surface biotinylation assay

Following 48 h of incubation, transiently transfected HEK293 cells were treated with EZlinkTM Sulfo-NHS-SS-Biotin (Thermo Scientific, Rockford, Illinois, USA) 0.5 mg/ml in cold 1X PBS for 15 min at 4 °C. Subsequently, the cells were washed twice with 200 mM glycine in cold 1X PBS and twice with cold 1X PBS to inactivate and remove the excess biotin, respectively. The cells were then lysed with 1X lysis buffer (50 mM HEPES pH 7.4; 150 mM NaCl; 1.5 mM $MgCl_2$; 1 mM EGTA pH 8.0; 10% Glycerol; 1% Triton X-100; 1X Complete Protease Inhibitor Cocktail (Roche, Mannheim, Germany)) for 1 h at 4 °C. Cell lysates were centrifuged at 16,000 g; 4 °C for 15 min. Two milligram of the supernatant was incubated with 50 μl Streptavidin Sepharose High Performance beads (GE Healthcare, Uppsala, Sweden) for 2 h at 4 °C, and the remaining supernatant was kept as the input. The beads were subsequently washed five times with 1X lysis buffer before elution with 50 μl of 2X NuPAGE sample buffer (Invitrogen, Carlsbad, California, USA) plus 100 mM DTT at 37 °C for 30 min. These biotinylated fractions were analyzed as TRPM4 expressed at the cell surface. The input fractions, analyzed as total expression of TRPM4, were resuspended with 4X NuPAGE Sample Buffer plus 100 mM DTT to give a concentration of 1 mg/ml and incubated at 37 °C for 30 min.

2.7. Western blot experiments

Protein samples were analyzed on 9% polyacrylamide gels, transferred with the TurboBlot dry blot system (Biorad, Hercules, CA, USA) and detected with anti-TRPM4 (generated by Pineda, Berlin, Germany), anti α-actin A2066 (Sigma-Aldrich, St. Louis, Missouri, USA) antibodies using SNAP i.d. (Millipore, Billerica, MA, USA). The anti-TRPM4 antibody was generated by Pineda (Berlin, Germany) using the following peptide sequence: NH2-CRDKRESDSERLKRTSQKV-CONH2. A fraction of the antisera, which was subsequently used in this study, was then affinity purified.

2.8. Cellular electrophysiology

For patch-clamp experiments in whole-cell configuration, glass pipettes (tip resistance, 1.5–3 MΩ) were filled with an intracellular solution containing (in mM): 100 CsAsp, 20 CsCl, 4 Na_2ATP, 1 $MgCl_2$, 10 EGTA, and 10 HEPES. The pH was adjusted to 7.20 with CsOH, and the free Ca^{2+} concentration at 100 μM with $CaCl_2$ using WEBMAXCLITE program (http://www.stanford.edu/~cpatton/downloads.htm). Access resistance ranges was from 3 to 5 MΩ. Extracellular solution contained (in mM): 156 NaCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 6 CsCl, 10 glucose and 10 HEPES. The pH was adjusted to 7.40 with NaOH. Patch-clamp recordings were carried-out in the whole-cell configuration at room temperature (23–25 °C). TRPM4 currents were investigated using a ramp protocol. The holding potential was −60 mV. The 400 ms increasing ramp from −100 to +100 mV ends with a 300 ms step at +100 mV then 300 ms at −100 mV. A new ramp was performed every 2 s. Before seal formation, liquid junction potential was compensated to keep the baseline at 0 mV. Using a Digidata 1440 A analog-digital interface (Axon Instruments,Inc.), currents were filtered at 5 kHz and the sampling frequency was at 50 kHz. Current densities were obtained by dividing the peak current recorded at −100 mV by the cell capacitance (17 ± 2 pF and 16 ± 1 pF, respectively transfected with WT and I376T-TRPM4 channels). Of note, capacitances and series resistances were not compensated.

2.9. Data analysis and statistical methods

Currents were analyzed with Clampfit software (Axon Instruments, Inc). Data were analyzed using a combination of pClamp10, Excel (Microsoft) and Prism (Graphpad).

Comparisons between groups were performed with impaired two-tailed Student's t test. Data are expressed as mean + SEM. A p-value <0.05 was considered significant.

3. Results

3.1. Mutational screening

Ninety-five patients with PCCD were recruited through the French National Referral Center for Sudden Cardiac Death as previously described [25]. Nineteen genes known or suspected to be involved in conduction defects were sequenced in these patients using the HaloPlex™ System, resulting in a mean coverage depth of 578× per sample: *SCN5A*, *SCN1B*, *TRPM4*, *GJA5* and *LMNA* that have already been associated with isolated cardiac conduction defects together with *GJA1*, *GJC1*, *SCN10A*, *NKX2-5*, *TBX5*, *SNTA1*, *PRKAG2*, *RYR2*, *EMD*, *BMP2*, *BMPR1A*, *GATA4*, *MSX2* and *TNNI3K*. A graphical representation of the mean coverage obtained for the 5 major genes is provided in Supplemental Fig. 1.

When selecting only genetic variants with a potential pathogenicity as predicted by Variant Effect Predictor (see methods) and an MAF below 1% in public databases, we identified a total of 45 variants in 43 patients: 11 novel variants and 34 rare variants (see Supplemental Table 1). Among these variants, 13 have already been associated with cardiac pathologies such as the Brugada syndrome and cardiac