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Rapid, sensitive and inexpensive detection of *SCN5A* genetic variations by high resolution melting analysis

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Abstract

Objectives: *SCN5A* mutations lead to a wide spectrum of cardiovascular disorders. Due to large cohorts to investigate and the large gene size, mutational screening must be performed using an extremely sensitive and specific scanning method.

Design and methods: High Resolution Melting (HRM) analysis was developed for *SCN5A* mutation detection using control DNAs and DNAs carrying previously identified gene variants. A cohort of 40 patients was further screened. To evaluate HRM sensitivity, this cohort was also screened using an optimized DHPLC methodology.

Results: All gene variants detected by DHPLC were also readily identified as abnormal by HRM analysis. Mutations were identified for 5 patients. Complete molecular SCN5A investigation was completed two times faster and cheaper than using DHPLC strategy.

Conclusions: HRM analysis represents an inexpensive, highly sensitive and high-throughput method to allow identification of *SCN5A* gene variants. Identification of more *SCN5A* mutations could provide new insights into the pathophysiology of SCN5A-linked diseases syndromes.
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Keywords: Mutations; Cardiovascular disorders; High resolution melting; DHPLC; Polymorphisms; SCN5A

Introduction

Voltage-gated sodium (Na^+) channels, encoded by the SCNxA family of genes, are transmembrane proteins responsible for the rising phase of the action potential in nerve and muscle cells [1]. Due to this central role in excitability, it is not surprising that inherited mutations in *SCN5A* [MIM#: 600163, Swiss-Prot: Q14524], the gene encoding the Na^+ channel α-subunit expressed in the human heart, could lead to a wide spectrum of cardiovascular disorders. These include Long-QT syndrome [LQTS] which has a prevalence estimated at about 1:5000 persons, Brugada syndrome [BS], dilated cardiomyopathy [DCM], progressive cardiac conduction disease [PCCD], sick sinus syndrome [SSS], idiopathic ventricular fibrillation, and more complex overlapping phenotypes representing combinations of LQTS, conduction system disease, and Brugada syndrome [2–9]. Mutations or rare variants in *SCN5A* may also predispose patients, with or without underlying heart disease, to atrial fibrillation [AF] which is the most common cardiac arrhythmia in clinical practice [10,11]. Finally, 2–5% of cases diagnosed as sudden infant death syndrome [SIDS], the leading cause of mortality in the first year of life in the postneonatal period, carry functionally significant *SCN5A* genetic variants [12–14].

Consequently, in medical pratice, mutational screening on SCN5A of patients with syndromes quoted above is crucial for proper management of patients and affected families. Molecular analysis of these patients is however challenging owing to the size of this gene, presence of a large spectrum of mutations and the occurrence of numerous polymorphisms (for more information on previously reported mutations, see http://pc4.fsm.it:81/cardmoc/). To date, most of the mutational screening in patients was performed either by direct sequencing or by DHPLC/sequencing [15–20]. These methods for large-scale detection of

Abbreviations: LQTS, Long-QT syndrome; SIDS, Sudden Infant Death Syndrome; AF, Atrial Fibrillation; DCM, Dilated Cardiomyopathy; HRMA, High Resolution Melting Analysis; BS, Brugada Syndrome; DHPLC, Denaturing High-Performance Liquid Chromatography.

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Table 1
HRM conditions for mutation scanning of SCN5A gene

Exon		Forward primer (5′→3′)	Reverse primer (5′→3′)	Size (bp)	T°a [a] (°C)	$MgCl_2$ (mM)	HRM conditions		
							Melting T° ranges		Mutation threshold confidence percentage
							Leading range	Trailing range	
1		GCCGCTGAGCCTGCGCCCAGT	GGAAAGTTGGGCGGCGGCAG	172	70–60	2	86.3–87.7	92.5–94	90
							84–86.3	92.5–94.9	90
2	2a	GAATCAGGCCCATTGTCTGT	TCTCCTGCAAGGTGGTTGA	205	70–60	3	84.2–85.1	90.2–91	95
							85.9–86.3	88–88.4	95
							87.1–87.5	89.4–89.9	95
	2b	CTGGCAGCCATCGAGAAG	GTAGGCAGGGCTGGAGGT	244	70–60	2	82.4–84.8	92–95	94
							85.6–86.3	88.8–89.2	94
							88.2–88.6	91.2–91.8	94
3		AGTCCAAGGGCTCTGAGCCAA	GGTACTCAGCAGGTATTAACTGCAA	228	70–60	3	81.2–82.7	86.7–88.2	95
							77.7–80.7	86.3–90	95
4		TGGCCTGGCAGTGATGACCCC	AAAGGAAGGGAGGGGGCCACGTG	231	70–60	3	83–83.7	87.7–88.3	95
							82–84	90.7–93	95
5		TCACTCCACGTAAGGAACCTG	ATGTGGACTGCAGGGAGGAAGC	324	70–60	1.5	83–84	88–90	90
							80.8–83	90–92.2	90
6		CCCCACCCCCTTTCCTCCTCTGA	AGGGTTGCCTTGGCTCCCAGGTG	251	70–60	2.5	75.9–77.1	87–88.2	90
							76.1–78.5	87–89.4	90
7		CCACCAGTGGAGCACAGAG	GGTCTGCTGGTCTCACAAAG	344	70–60	3	82.9–84	90.8–91.9	93
							82.8–84.8	91.6–93.4	94
8		CGAGTGCCCCTCACCAGCATG	GGAGACTCCCCTGGCAGGACAA	152	70–60	3	80.5–81.5	87.2–88.2	90
							77.8–80.2	87.7–90	90
9		GGGAGACAAGTCCAGCCCAGCAA	AGCCCACACTTGCTGTCCCTTG	262	70–60	3	86.8–87.5	90.2–90.8	90
							82.1–84.1	92–94	90
10		CCAGAAGGGGCCCCAGTGAGG	AGGCTCCTCGGTGGCACTGCTCA	274	70–65	2	83.3–84.2	87.1–88	90
							84–84.6	86.3–86.7	95
11		AAACGTCCGTTCCTCCACTCT	AACCCACAGCTGGGATTACCATT	226	70–60	3	82.8–83.9	86.9–88	90
							79.4–81.4	87.5–89.4	90
12	12a	GCCAGTGGCTCAAAAGACAGGCT	CCTGGGCACTGGTCCGGCGCA	280	60	3	80.5–85	91.2–96	94
							87.7–88.7	90.6–93	97
	12b	AGCGGGGGAGAGCGAGAG	TGTGGTGCCTGCATCTCG	294	65–55	2.5	87.4–89.3	93.2–94.9	95
							88.6–89.7	91.5–91.8	95
13		CCCTTTTCCCCAGCTGACGCAAA	GTCTAAAGCAGGCCAAGACAAATG	283	70–60	2	84.4–85.13	92.2–93	90
							84.6–85.1	88.7–89.1	95
							88.5–88.9	91.4–91.9	90
14		TCCTGGAAGGTATTCCAGTTAC	CTTACCCATGAAGGCTGTGC	394	70–60	3.5	84.8–85.8	89.7–90.7	90
							83.6–85.2	90–91.6	90
15		GCCCCTGCCACAGCAAGAGTCAA	GCCTTCCACACCCCCCACCAT	309	65–55	3.2	82.1–83.1	90–91.2	90
							84.6–85.5	86.9–87.6	95
							86.6–87.3	89.2–90	90
16	16a	GAGCCAGAGACCTTCACAAGGTCCCCT	GCCAAAGAGCTGCATGCCCACCA	195	70–60	2.5	83.2–83.8	87.9–88.6	90
							81.6–83	88.6–90	92
	16b	GGCACTGGGGAACCTGACACTG	GGATGGTGTGTGTGTGGCCCTTG	307	70–60	2.5	82.8–83.7	88.8–89.7	93
17	17a	GGGACTGGATGGCTTGGCATGGT	CGGGGAGTAGGGGGTGGCAATG	306	70–65	2	81.9–82.9	93.6–94.5	90
							85.2–85.8	88.4–89.1	95
							88.3–88.8	92.3–92.9	90
	17b	GCCCAGGGCCAGCTGCCCAGCT	CTGTATATGTAGGTGCCTTATACATG	283	70–60	2	85.6–86.6	92.7–93.7	90
							84.8–86.4	92.6–94.2	90
18		AGGGTCTAAACCCCCAGGGTCA	CCCAGCTGGCTTCAGGGACAAA	280	65–55	3.2	87.5–89	92–93.5	95
							87.9–88.7	90.9–91.7	95
19		GCTGCTACTCAGCCCACACT	TCTGGGTGGAACTGAGGCTA	226	70–60	3	85.7–86.5	90.9–91.8	90
							84.1–85.6	90.4–91.9	90
20		ACAGGCCCTGAGGTGGGCCTGA	TGACCTGACTTTCCAGCTGGAGA	287	65–55		86–87.1	91.5–92.6	92
							87.3–87.9	90.8–91.5	92
21		ATCGGCAGTGGTCCAGGCTT	CTCCGCCTCAGCTCCTTCTC	297	65–55	3	83.7–84.6	88.6–89.6	90
							81.9–83.9	89.2–91.1	90
22		GCCTACTGTCTGTCCCCAAC	CACTCCCTGGTGGGAAGG	225	70–60	3	84.3–85.2	89.7–90.6	90
							80.9–82.8	90.6–92.6	90
23	23a	GGTCTTGAAAAGGGCATGTG	GCAAGTCTCCCTCTGTCTGG	216	70–60	2	82.6–84.4	88.5–90.3	90
							83.8–94.5	87.8–88.5	90
	23b	GGAAGTTTGGGAGGTGCAT	CCATTGGGAGGAAGGAAGTC	212	70–60	2	80.1–81.1	86.4–87.2	92

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