

Online Appendix for the following *JACC* article

TITLE: *SCN5A* Mutations Associate With Arrhythmic Dilated Cardiomyopathy and Commonly Localize to the Voltage-Sensing Mechanism

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APPENDIX

Table 1. Conservation Analysis of Residues T220, R222 of Domain I, S4, and R814 of Domain II, S4*

| Protein | NCBI Identifier | Location | | S4 Sequence | | | | | | | | | | | | | | | | | | | | | | | |
|--------------|-----------------|----------------------|------------|-------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|---|---|---|
| KHACN4 | Q9Y3Q4 | S4 | 369 | | T | K | I | L | S | L | L | R | L | L | R | L | S | R | L | I | R | Y | I | H | | | |
| BCNG1 | O60741 | S4 | 249 | | T | K | I | L | S | L | L | R | L | L | R | L | S | R | L | I | R | Y | I | H | | | |
| BCNG2 | Q9UL51 | S4 | 318 | | T | K | I | L | S | L | L | R | L | L | R | L | S | R | L | I | R | Y | I | H | Q | | |
| Kv11.1 | Q12809 | S4 | 521 | | L | L | K | T | A | R | L | L | R | L | V | R | V | A | R | K | L | D | R | Y | | | |
| Kv11.2 | Q9H252 | S4 | 371 | | L | L | K | T | A | R | L | L | R | L | V | R | V | A | R | K | L | D | R | Y | | | |
| KvH2C | NP_742054 | S4 | 181 | | L | L | K | T | A | R | L | L | R | L | V | R | V | A | R | K | L | D | R | Y | | | |
| Kv10.1 | O95259 | S4 | 350 | | S | L | K | V | V | R | L | L | R | L | G | R | V | A | R | K | L | D | H | Y | | | |
| Kv10.2 | Q8NCM5 | S4 | 320 | | S | L | K | V | V | R | L | L | R | L | G | R | V | A | R | K | L | D | H | Y | L | | |
| SCN11A | Q9UI33 | Domain IV, S4 | 1462 | | F | R | I | V | R | L | A | R | I | G | R | I | L | R | L | V | R | A | A | R | | | |
| SCN10A | Q9Y5Y9 | Domain IV, S4 | 1573 | | F | R | V | I | R | L | A | R | I | G | R | I | L | R | L | I | R | A | A | K | | | |
| SCN4A | P35499 | Domain IV, S4 | 1447 | | F | R | V | I | R | L | A | R | I | G | R | V | L | R | L | I | R | G | A | K | | | |
| SCN5A | Q14524 | Domain IV, S4 | 1622 | | F | R | V | I | R | L | A | R | I | G | R | I | L | R | L | I | R | G | A | K | | | |
| SCN1A | P35498 | Domain IV, S4 | 1636 | | F | R | V | I | R | L | A | R | I | G | R | I | L | R | L | I | K | G | A | K | | | |
| SCN2A | Q99250 | Domain IV, S4 | 1626 | | F | R | V | I | R | L | A | R | I | G | R | I | L | R | L | I | K | G | A | K | | | |
| SCN3A | Q9NY46 | Domain IV, S4 | 1621 | | F | R | V | I | R | L | A | R | I | G | R | I | L | R | L | I | K | G | A | K | | | |
| SCN8A | Q9UQD0 | Domain IV, S4 | 1617 | | F | R | V | I | R | L | A | R | I | G | R | I | L | R | L | I | K | G | A | K | | | |
| SCN9A | Q15858 | Domain IV, S4 | 1610 | | F | R | V | I | R | L | A | R | I | G | R | I | L | R | L | V | K | G | A | K | | | |
| CACNA1G | O43497 | Domain III, S4 | 1379 | | L | R | V | L | R | L | L | R | T | L | R | P | L | R | V | I | S | R | A | Q | | | |
| CACNA1I | Q9P0X4 | Domain III, S4 | 1271 | | L | R | V | L | R | L | R | T | L | R | P | L | R | V | I | S | R | A | P | | | | |
| CACNA1S | Q13698 | Domain III, S4 | 893 | | V | K | I | L | R | V | L | R | V | L | R | P | L | R | A | I | N | R | A | K | | | |
| CACNA1C | Q13936 | Domain III, S4 | 1014 | | V | K | I | L | R | V | L | R | V | L | R | P | L | R | A | I | N | R | A | K | | | |
| CACNA1G | O43497 | Domain I, S4 | 176 | | F | S | A | V | R | T | V | R | V | L | R | P | L | R | A | I | N | R | V | P | | | |
| CACNA1S | Q13698 | Domain I, S4 | 161 | | V | K | A | L | R | A | F | R | V | L | R | P | L | R | L | V | S | G | V | P | | | |
| CACNA1C | Q13936 | Domain I, S4 | 233 | | V | K | A | L | R | A | F | R | V | L | R | P | L | R | L | V | S | G | V | P | | | |
| CACNA1I | Q9P0X4 | Domain I, S4 | 174 | | L | S | A | I | R | T | V | R | V | L | R | P | L | K | A | I | N | R | V | P | | | |
| CACNA1A | O00555 | Domain I, S4 | 191 | | L | R | T | L | R | A | V | R | V | L | R | P | L | K | L | V | S | G | I | P | | | |
| CACNA1E | NP_000712 | Domain I, S4 | 186 | | L | R | T | L | R | A | V | R | V | L | R | P | L | K | L | V | S | G | I | P | | | |
| SCN1A | P35498 | Domain III, S4 | 1315 | | | | | L | R | T | L | R | A | L | R | P | L | R | A | L | S | R | F | E | G | M | R |
| SCN5A | Q14524 | Domain III, S4 | 1296 | | | | | L | R | T | L | R | A | L | R | P | L | R | A | L | S | R | F | E | G | M | R |
| SCN2A | Q99250 | Domain III, S4 | 1299 | | | | | L | R | T | L | R | A | L | R | P | L | R | A | L | S | R | F | E | G | M | R |
| SCN3A | Q9NY46 | Domain III, S4 | 1303 | | | | | L | R | T | L | R | A | L | R | P | L | R | A | L | S | R | F | E | G | M | R |
| SCN4A | P35499 | Domain III, S4 | 1122 | | | | | L | R | T | L | R | A | L | R | P | L | R | A | L | S | R | F | E | G | M | R |
| SCN8A | Q9UQD0 | Domain III, S4 | 1295 | | | | | L | R | T | L | R | A | L | R | P | L | R | A | L | S | R | F | E | G | M | R |
| SCN9A | Q15858 | Domain III, S4 | 1289 | | | | | L | R | T | L | R | A | L | R | P | L | R | A | L | S | R | F | E | G | M | R |
| SCN10A | Q9Y5Y9 | Domain III, S4 | 1243 | | | | | L | R | T | L | R | A | L | R | P | L | R | A | L | S | R | F | E | G | M | R |
| SCN11A | Q9UI33 | Domain III, S4 | 1140 | | | | | F | R | T | L | R | A | L | R | P | L | R | A | L | S | Q | F | E | G | M | K |
| CACNA1A | O00555 | Domain III, S4 | 1342 | | I | K | S | L | R | V | L | R | V | L | R | P | L | K | T | I | K | R | L | P | | | |
| CACNA1E | NP_000712 | Domain III, S4 | 1254 | | I | K | S | L | R | V | L | R | V | L | R | P | L | K | T | I | K | R | L | P | | | |
| SCN7A | Q01118 | Domain I, S4 | 204 | | T | L | Q | T | A | R | T | L | R | I | L | K | I | I | P | L | N | Q | G | L | | | |
| SCN1A | P35498 | Domain I, S4 | 214 | | A | L | R | T | F | R | V | L | R | A | L | K | T | I | S | V | I | P | G | L | | | |
| SCN2A | Q99250 | Domain I, S4 | 215 | | A | L | R | T | F | R | V | L | R | A | L | K | T | I | S | V | I | P | G | L | | | |
| SCN3A | Q9NY46 | Domain I, S4 | 214 | | A | L | R | T | F | R | V | L | R | A | L | K | T | I | S | V | I | P | G | L | | | |
| SCN4A | P35499 | Domain I, S4 | 217 | | A | L | R | T | F | R | V | L | R | A | L | K | T | I | T | V | I | P | G | L | | | |
| SCN5A | Q14524 | Domain I, S4 | 217 | | A | L | R | T | F | R | V | L | R | A | L | K | T | I | S | V | I | S | G | L | | | |
| SCN8A | Q9UQD0 | Domain I, S4 | 218 | | A | L | R | T | F | R | V | L | R | A | L | K | T | I | S | V | I | P | G | L | | | |
| SCN9A | Q15858 | Domain I, S4 | 212 | | A | L | R | T | F | R | V | L | R | A | L | K | T | I | S | V | I | P | G | L | | | |
| SCN10A | Q9Y5Y9 | Domain I, S4 | 213 | | G | L | R | T | F | R | V | L | R | A | L | K | T | V | S | V | I | P | G | L | | | |
| SCN11A | Q9UI33 | Domain I, S4 | 220 | | P | L | R | T | F | R | V | F | R | A | L | K | A | I | S | V | V | S | R | L | | | |
| SCN11A | Q9UI33 | Domain II, S4 | 668 | | F | L | R | S | F | R | V | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |
| SCN5A | Q14524 | Domain II, S4 | 806 | | V | L | R | S | F | R | L | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |
| SCN1A | P35498 | Domain II, S4 | 857 | | V | L | R | S | F | R | L | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |
| SCN2A | Q99250 | Domain II, S4 | 848 | | V | L | R | S | F | R | L | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |
| SCN3A | Q9NY46 | Domain II, S4 | 849 | | V | L | R | S | F | R | L | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |
| SCN4A | P35499 | Domain II, S4 | 667 | | V | L | R | S | F | R | L | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |
| SCN7A | Q01118 | Domain II, S4 | 593 | | L | L | R | L | F | R | M | L | R | I | F | K | L | G | K | Y | W | P | T | F | | | |
| SCN8A | Q9UQD0 | Domain II, S4 | 842 | | V | L | R | S | F | R | L | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |
| SCN9A | Q15858 | Domain II, S4 | 833 | | V | L | R | S | F | R | L | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |
| SCN10A | Q9Y5Y9 | Domain II, S4 | 754 | | V | L | R | S | F | R | L | L | R | V | F | K | L | A | K | S | W | P | T | L | | | |

Table 1, continued

| Protein | NCBI Identifier | Location | | S4 Sequence | | | | | | | | | | | | | | | | | | | | | | | | | |
|-----------|-----------------|---------------|------|-------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CACNA1A | O00555 | Domain II, S4 | 579 | | | I | S | V | L | R | A | L | R | L | L | R | I | F | K | V | T | K | Y | W | A | | | | |
| CACNA1E | NP_000712 | Domain II, S4 | 568 | | | I | S | V | L | R | A | L | R | L | L | R | I | F | K | I | T | K | Y | W | A | | | | |
| CACNA1S | Q13698 | Domain II, S4 | 524 | | | I | S | V | L | R | C | I | R | L | L | R | I | F | K | I | T | K | Y | W | T | | | | |
| CACNA1C | Q13936 | Domain II, S4 | 616 | | | I | S | V | L | R | C | V | R | L | L | R | I | F | K | I | T | R | Y | W | N | | | | |
| Kv1.2 | NP_000710.4 | S4 | 293 | | | L | R | V | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| Kv1.1 | NP_000208 | S4 | 291 | | | L | R | V | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| Kv1.3 | NP_002223 | S4 | 311 | | | L | R | V | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| Kv1.5 | NP_002225 | S4 | 398 | | | L | R | V | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| Kv1.6 | NP_002226 | S4 | 340 | | | L | R | V | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| Kv1.7 | NP_114092 | S4 | 276 | | I | L | R | V | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| Kv1.10 | NP_005540 | S4 | 334 | I | | L | R | I | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| Kv1.4 | P15385 | S4 | 445 | | | L | R | I | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| Kv1.4 | NP_002224 | S4 | 443 | | | L | R | I | I | R | L | V | R | V | F | R | I | F | K | L | S | R | H | S | | | | | |
| KvSHAL3-2 | NP_751948 | S4 | 286 | | | F | V | T | L | R | V | F | R | V | F | R | I | F | K | F | S | R | H | S | | | | | |
| KvSHAL3-1 | NP_004971 | S4 | 286 | | | F | V | T | L | R | V | F | R | V | F | R | I | F | K | F | S | R | H | S | | | | | |
| KvSHAL2 | NP_036413 | S4 | 293 | | | F | V | T | L | R | V | F | R | V | F | R | I | F | K | F | S | R | H | S | | | | | |
| KvSHAL1 | AAH45659 | S4 | 289 | | | S | G | A | F | V | T | L | R | V | F | R | I | F | K | F | S | R | H | S | | | | | |
| Kv3.4 | Q03721 | S4 | 345 | | | L | R | V | V | R | F | V | R | I | L | R | I | F | K | L | T | R | H | F | | | | | |
| KvDRect | NP_002243 | S4 | 291 | | | I | L | R | L | M | R | I | F | R | I | L | K | L | A | R | H | S | | | | | | | |
| KvG3-1 | AAH71558 | S4 | 288 | | | L | R | V | L | R | M | M | R | I | F | W | V | I | K | L | A | R | H | F | | | | | |
| CACNA1I | Q9P0X4 | Domain II, S4 | 727 | | | L | S | V | L | R | T | F | R | L | R | V | L | K | L | V | R | F | M | P | | | | | |
| CACNA1G | O43497 | Domain II, S4 | 833 | | | L | S | V | L | R | T | F | R | L | M | R | V | L | K | L | V | R | F | L | P | | | | |
| CACNA1C | Q13936 | Domain IV, S4 | 1373 | | | I | T | F | F | R | L | F | R | V | M | R | L | V | K | L | L | S | R | G | E | | | | |
| CACNA1G | O43497 | Domain IV, S4 | 1708 | | | I | R | I | M | R | V | L | R | I | A | R | V | L | K | L | L | K | M | A | V | | | | |
| CACNA1I | Q9P0X4 | Domain IV, S4 | 1584 | | | I | R | I | M | R | V | L | R | I | A | R | V | L | K | L | L | K | M | A | T | | | | |
| CACNA1A | O00555 | Domain IV, S4 | 1657 | | | L | S | F | L | R | L | F | R | A | A | R | L | I | K | L | L | R | Q | G | Y | | | | |
| CACNA1E | NP_000712 | Domain IV, S4 | 1572 | | | M | S | F | L | K | L | F | R | A | A | R | L | I | K | L | L | R | Q | G | Y | | | | |
| KvSHAL1 | AAH46629 | S4 | 342 | | V | L | R | V | L | R | A | L | R | I | L | Y | V | M | R | L | A | R | H | S | | | | | |
| Kv7.1 | P51787 | S4 | 228 | | | R | G | I | R | F | L | Q | I | L | R | M | L | H | V | D | R | Q | G | G | T | W | K | L | V |
| Kv7.2 | O88943 | S4 | 198 | | | R | S | L | R | F | L | Q | I | L | R | M | I | R | M | D | R | R | G | G | T | W | K | L | V |
| Kv7.4 | P56696 | S4 | 202 | | | R | S | M | R | F | L | Q | I | L | R | M | V | R | M | D | R | R | G | G | T | W | F | G | I |
| TRPM1 | NP_002411 | S4 | 848 | | | G | R | V | I | Y | C | V | D | I | I | F | W | Y | I | R | V | L | D | I | F | T | V | | |
| TRPM2 | O94759 | S4 | 897 | | | G | R | V | I | L | S | L | D | F | I | L | F | C | L | R | L | M | H | I | F | G | V | | |
| TRPM3 | NP_002411 | S4 | 996 | | | G | R | V | I | Y | C | V | N | I | I | Y | W | Y | I | R | L | L | D | I | F | T | I | | |
| TRPM4 | NP_060106 | S4 | 893 | | | G | R | T | V | L | C | I | D | F | M | V | F | T | V | R | L | L | H | I | F | A | V | | |
| TRPM5 | NP_055370 | S4 | 833 | | | G | R | T | V | L | A | M | D | F | M | V | F | T | V | R | L | L | H | I | F | A | V | | |
| TRPM6 | Q9BX84 | S4 | 940 | | | G | R | L | I | Y | C | I | D | I | I | F | W | F | S | R | L | L | D | F | L | A | V | | |
| TRPM7 | Q96QT4 | S4 | 963 | | | G | R | L | I | Y | C | L | N | I | I | F | W | Y | V | R | L | L | D | F | F | T | | | |
| TRPM8 | Q7Z2W7 | S4 | 830 | | | G | R | V | I | F | C | L | D | Y | I | I | F | T | L | R | L | I | H | I | | | | | |

*BLAST alignments of S4 transmembrane segments among homologous proteins

demonstrate sequence conservation of positions T220, R222, and R814 in *SCN5A*.

Relevant residues and segments of human *SCN5A* are in bold typeface and bracketed for easier identification. Colors were randomly selected and assigned to different amino acids to facilitate alignment.

Table 2. Conservation Analysis of SCN5A Residues E446 and F1520*

| Species | Protein | NCBI Identifie | E446 Conservation | | | | | | | | F1520 Conservation | | | | | | | | | | | | | | | |
|-------------------|---------|----------------|-------------------------------|---|---|---|---|---|----------|---|----------------------------------|---|---|---|----------------------------------|---|----------|---|---|---|----------|---|---|---|---|---|
| Human | SCN5A | Q14524 | 441 | L | K | K | E | H | E | A | L | T | I | R | 1515 | N | K | Y | Q | G | F | I | F | D | I | V |
| Mus Musculus | SCN5A | 84875498 | 441 | L | K | K | E | H | E | A | L | T | I | R | 1518 | N | K | Y | Q | G | F | I | F | D | I | V |
| Canis Lupus | SCN5A | 50950233 | 441 | L | K | K | E | H | E | A | L | T | I | R | 1512 | N | K | Y | Q | G | F | L | F | D | I | V |
| Bos Taurus | SCN5A | 27806135 | 444 | L | K | K | E | Q | E | A | L | A | I | R | 1520 | N | K | Y | Q | G | F | I | F | D | I | V |
| Gallus Gallus | SCN5A | 118085545 | 439 | L | K | K | E | Q | E | A | L | A | A | K | 1539 | N | K | Y | Q | G | F | I | F | D | V | V |
| Pan Troglodytes | SCN5A | 114586083 | 441 | L | K | K | E | H | E | A | L | T | I | R | 1515 | N | K | Y | Q | G | F | I | F | D | I | V |
| Canis familiaris | SCN5A | 29691680 | 442 | L | K | K | E | H | E | A | L | T | I | R | 1512 | N | K | Y | Q | G | F | L | F | D | I | V |
| Rattus norvegicus | SCN5A | P15389.1 | 441 | L | K | K | E | H | E | A | L | T | I | R | 1517 | N | K | Y | Q | G | F | I | F | D | I | V |
| Human | SCN1A | P35498 | 451 | L | K | K | Q | Q | E | A | A | Q | Q | A | 1528 | N | K | F | Q | G | M | V | F | D | F | V |
| Human | SCN2A | Q99250 | 452 | L | K | K | Q | Q | E | E | A | Q | A | A | 1518 | N | K | F | Q | G | M | V | F | D | F | V |
| Human | SCN3A | Q9NY46 | 451 | L | K | K | Q | Q | E | E | A | Q | A | V | 1513 | N | K | F | Q | G | M | V | F | D | F | V |
| Human | SCN4A | P35499 | 472 | F | K | K | H | Q | E | E | L | E | K | A | 1340 | N | K | I | Q | G | M | V | Y | D | L | V |
| Human | SCN7A | Q01118 | 422 | L | Q | E | G | N | E | T | D | E | A | K | 1238 | N | K | L | Q | G | F | I | F | D | V | V |
| Human | SCN8A | Q9UQD0 | 439 | L | K | K | Q | Q | E | E | A | Q | A | A | 1509 | N | K | I | Q | G | I | V | F | D | F | V |
| Human | SCN9A | Q15858 | 430 | L | K | K | E | Q | E | E | A | E | A | I | 1502 | N | K | I | Q | G | C | I | F | D | L | V |
| Human | SCN10A | Q9Y5Y9 | 426 | L | R | K | E | Q | E | V | L | A | A | L | 1463 | N | K | F | Q | G | F | V | F | D | I | V |
| Human | SCN11A | Q9UI33 | 428 | L | K | E | E | K | E | A | L | V | A | M | 1353 | N | K | C | Q | G | L | V | F | D | I | V |
| Human | CAC1A | O00555 | 396 | I | S | K | A | E | E | V | I | L | A | E | Poor Alignment / No Conservation | | | | | | | | | | | |
| Human | CAC1B | Q00975 | 392 | I | F | K | A | E | E | V | M | L | A | E | Poor Alignment / No Conservation | | | | | | | | | | | |
| Human | CAC1C | Q13936 | No alignment at this position | | | | | | | | Poor Alignment / No Conservation | | | | | | | | | | | | | | | |
| Human | CAC1D | Q01668 | No alignment at this position | | | | | | | | Poor Alignment / No Conservation | | | | | | | | | | | | | | | |
| Human | CAC1E | Q15878 | No alignment at this position | | | | | | | | Poor Alignment / No Conservation | | | | | | | | | | | | | | | |
| Human | CAC1F | O60840 | No alignment at this position | | | | | | | | Poor Alignment / No Conservation | | | | | | | | | | | | | | | |
| Human | CAC1G | O43497.3 | No alignment at this position | | | | | | | | Poor Alignment / No Conservation | | | | | | | | | | | | | | | |
| Human | CAC1H | O95180 | No alignment at this position | | | | | | | | Poor Alignment / No Conservation | | | | | | | | | | | | | | | |
| Human | CAC1I | Q9P0X4.1 | No alignment at this position | | | | | | | | Poor Alignment / No Conservation | | | | | | | | | | | | | | | |
| Human | CAC1S | Q13698 | No alignment at this position | | | | | | | | 405 | N | K | I | I | Q | F | I | R | H | W | R | | | | |

*BLAST alignments of sequence immediately adjoining novel mutation sites E446 and F1520 in *SCN5A* demonstrate conservation among homologous, orthologous, and paralogous proteins. Pertinent positions within alignments are in boldface and bracketed for easier comparison.

Table 3. Conservation Analysis of Residues D1275 and V1279 of Domain III, S3, and D1595 of Domain IV, S3*

| Protein | NCBI Identifier | Location | | S3 Segment | | | | | | | | | | | | | | | | | | | |
|-----------|-----------------|----------------|------|------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CACNA1S | Q13698 | Domain II, S3 | 495 | I | F | N | R | F | D | C | F | V | V | C | S | G | I | L | E | I | L | L | V |
| CACNA1C | Q13936 | Domain II, S3 | 587 | L | F | N | R | F | D | C | F | V | V | C | G | G | I | L | E | T | I | L | V |
| CACNA1A | O00555 | Domain II, S3 | 550 | S | F | N | C | F | D | C | G | V | I | I | G | S | I | F | E | V | I | W | A |
| CACNA1E | NP_000712 | Domain II, S3 | 539 | S | F | N | C | F | D | F | G | V | T | V | G | S | I | F | E | V | V | W | A |
| CACNA1S | Q13698 | Domain III, S3 | 867 | Y | F | N | M | L | D | L | L | V | V | A | S | L | I | S | M | G | L | E | S |
| CACNA1C | Q13936 | Domain III, S3 | 988 | Y | F | N | I | L | D | L | L | V | V | S | V | S | L | I | S | F | G | I | Q |
| CACNA1G | O43497 | Domain II, S3 | 806 | P | Y | N | I | F | D | G | V | I | V | V | I | S | V | W | E | I | V | G | Q |
| CACNA1I | Q9P0X4 | Domain II, S3 | 703 | P | Y | N | I | F | D | S | I | I | V | I | I | S | I | W | E | I | V | G | Q |
| KvSHAL1 | AAH45659 | S3 | 264 | M | S | L | I | D | V | V | A | I | L | P | Y | Y | I | G | L | V | P | K | N |
| KvSHAL3-2 | NP_751948 | S3 | 259 | M | S | I | I | D | V | V | A | I | M | P | Y | Y | I | G | L | V | M | T | N |
| KvSHAL3-1 | NP_004971 | S3 | 259 | M | S | I | I | D | V | V | A | I | M | P | Y | Y | I | G | L | V | M | T | N |
| KvSHAL2 | NP_036413 | S3 | 262 | M | S | I | I | D | V | V | A | I | L | P | Y | Y | I | G | L | V | M | T | D |
| Kv1.2 | NP_000710.4 | S3 | 255 | I | M | N | I | D | I | V | A | I | I | P | Y | F | I | T | L | G | T | E | L |
| Kv1.1 | NP_000208 | S3 | 254 | I | M | N | F | I | D | I | V | A | I | I | P | Y | F | I | T | L | G | T | E |
| Kv1.3 | NP_002223 | S3 | 276 | I | M | N | L | I | D | I | V | A | I | I | P | Y | F | I | T | L | G | T | E |
| Kv1.4 | NP_002224 | S3 | 404 | M | N | I | I | D | I | V | S | I | L | P | Y | F | I | T | L | G | T | E | L |
| Kv1.5 | NP_002225 | S3 | 356 | M | N | I | I | D | V | V | A | I | F | P | Y | F | I | T | L | G | T | E | L |
| Kv1.6 | NP_002226 | S3 | 296 | M | N | I | I | D | L | V | A | I | F | P | Y | F | I | T | L | G | T | E | L |
| Kv1.7 | NP_114092 | S3 | 242 | M | N | L | I | D | F | V | A | I | L | P | Y | F | V | A | L | G | T | E | L |
| Kv1.10 | NP_005540 | S3 | 304 | M | N | I | I | D | I | I | S | I | I | P | Y | F | A | T | L | I | T | E | L |
| KvDRect | NP_002243 | S3 | 255 | L | N | I | I | D | F | V | S | I | I | P | F | Y | A | T | L | A | V | D | T |
| Kv3.4 | Q03721 | S3 | 314 | L | N | I | I | D | F | V | A | I | L | P | F | Y | L | E | V | G | L | S | G |
| TRPM7 | Q96QT4 | S3 | 924 | F | N | I | S | D | T | I | A | I | I | S | F | F | I | G | F | G | L | R | F |
| ADPKD2 | Q13563 | S3 | 506 | F | W | N | C | L | D | V | V | I | V | V | L | S | V | V | A | I | G | I | N |
| PKD2L-1 | Q9P0L9 | S3 | 385 | I | W | N | I | L | D | L | V | V | I | L | L | S | I | V | A | V | G | F | H |
| TRPM1 | NP_002411 | S3 | 833 | y | W | N | I | T | D | L | V | A | I | S | T | F | M | I | G | A | I | L | R |
| TRPM2 | O94759 | S3 | 868 | W | N | K | L | D | V | G | A | I | L | L | F | V | A | G | L | T | C | R | L |
| TRPM3 | NP_002411 | S3 | 965 | W | N | V | T | D | L | I | A | I | L | L | F | S | V | G | M | I | L | R | L |
| TRPM4 | NP_060106 | S3 | 863 | W | N | Q | C | D | L | V | A | L | T | C | F | L | L | G | V | G | C | R | L |
| TRPM5 | NP_055370 | S3 | 807 | W | N | K | C | D | M | V | A | I | F | L | F | I | V | G | V | T | C | R | M |
| TRPM8 | Q7Z2W7 | S3 | 797 | W | N | V | M | D | T | L | G | L | F | Y | F | I | A | G | I | V | F | R | L |
| TRPC4 | Q9UBN4 | S3 | 434 | W | N | L | M | D | F | V | M | N | S | L | Y | L | A | T | I | S | L | K | I |
| TRPC6 | Q9UL62 | S3 | 435 | W | N | L | M | D | F | A | M | N | S | L | Y | L | A | T | I | S | L | K | I |
| TRPC5 | Q9Y210 | S3 | 525 | W | N | M | L | D | F | G | M | L | A | I | F | A | A | S | F | I | A | R | F |
| TRPC7 | Q9HCX4 | S3 | 471 | W | N | L | D | F | G | M | L | S | I | F | V | A | S | F | T | A | R | F | F |
| CACNA1E | NP_000712 | Domain IV, S3 | 1541 | T | W | N | I | F | D | F | I | T | V | I | G | S | I | T | E | I | I | L | T |
| CACNA1G | O43497 | Domain IV, S3 | 1674 | R | W | N | Q | L | D | L | A | I | V | L | L | S | I | M | G | I | T | L | E |
| CACNA1I | Q9P0X4 | Domain IV, S3 | 1557 | R | W | N | Q | L | D | L | A | I | V | L | L | S | V | M | G | I | T | L | E |
| CACNA1G | O43497 | Domain I, S3 | 151 | T | W | N | R | L | D | F | F | I | V | I | A | G | M | L | E | Y | S | L | D |
| CACNA1I | Q9P0X4 | Domain I, S3 | 149 | T | W | N | R | L | D | F | F | I | V | M | A | G | M | V | E | Y | S | L | D |
| SCN7A | Q01118 | Domain III, S3 | 999 | G | W | Y | R | L | D | F | V | V | V | I | V | F | C | L | S | L | I | G | K |
| SCN10A | Q9Y5Y9 | Domain II, S3 | 728 | K | W | N | I | F | D | C | I | I | V | T | V | S | L | L | E | L | G | V | A |
| SCN5A | Q14524 | Domain II, S3 | 780 | G | W | N | I | F | D | S | I | I | V | I | L | S | L | M | E | L | G | L | S |
| SCN1A | P35498 | Domain II, S3 | 831 | G | W | N | I | F | D | G | F | I | V | T | L | S | L | V | E | L | G | L | A |
| SCN2A | Q99250 | Domain II, S3 | 822 | G | W | N | I | F | D | G | F | I | V | S | L | S | L | M | E | L | G | L | A |
| SCN3A | Q9NY46 | Domain II, S3 | 823 | G | W | N | I | F | D | G | I | I | V | S | L | S | L | M | E | L | G | L | S |
| SCN4A | P35499 | Domain II, S3 | 641 | G | W | N | I | F | D | S | I | I | V | T | L | S | L | V | E | L | G | L | A |
| SCN7A | Q01118 | Domain II, S3 | 567 | G | W | N | I | F | D | S | M | I | V | F | H | G | L | I | E | L | C | L | A |
| SCN8A | Q9UQD0 | Domain II, S3 | 816 | G | W | N | I | F | D | G | F | I | V | S | L | S | L | M | E | L | S | L | |
| SCN9A | Q15858 | Domain II, S3 | 807 | G | W | N | I | F | D | S | L | I | V | T | L | S | L | V | E | L | F | L | A |
| SCN11A | Q9UI33 | Domain II, S3 | 640 | G | W | N | I | F | D | S | I | V | A | L | L | S | F | A | D | V | M | N | C |
| SCN1A | P35498 | Domain IV, S3 | 1602 | G | W | N | I | F | D | F | V | V | V | I | L | S | I | V | G | M | F | L | |
| SCN2A | Q99250 | Domain IV, S3 | 1592 | G | W | N | I | F | D | F | V | V | V | I | L | S | I | V | G | M | F | L | |
| SCN3A | Q9NY46 | Domain IV, S3 | 1587 | G | W | N | I | F | D | F | V | V | V | I | L | S | I | V | G | M | F | L | |
| SCN4A | P35499 | Domain IV, S3 | 1414 | G | W | N | I | F | D | F | V | V | V | I | L | S | I | V | G | L | A | L | |
| SCN8A | Q9UQD0 | Domain IV, S3 | 1583 | G | W | N | I | F | D | F | V | V | V | I | L | S | I | V | G | M | F | L | |
| SCN9A | Q15858 | Domain IV, S3 | 1576 | G | W | N | I | F | D | F | V | V | V | I | L | S | I | V | G | M | F | L | |
| SCN10A | Q9Y5Y9 | Domain IV, S3 | 1537 | G | W | N | V | F | D | F | I | V | V | V | L | S | I | A | S | L | I | F | |

Table 3, continued

| Protein | NCBI Identifier | Location | | S3 Segment | | | | | | | | | | | | | | | | | | | | |
|--------------|-----------------|-----------------------|-------------|------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|---|
| SCN2A | Q99250 | Domain I, S3 | 190 | P | W | N | W | L | D | F | T | V | I | T | F | A | Y | V | T | E | F | V | D | |
| SCN3A | Q9NY46 | Domain I, S3 | 189 | P | W | N | W | L | D | F | S | V | I | V | M | A | Y | V | T | E | F | V | S | |
| SCN4A | P35499 | Domain I, S3 | 191 | P | W | N | W | L | D | F | S | V | I | M | M | A | Y | L | T | E | F | V | D | |
| SCN7A | Q01118 | Domain I, S3 | 180 | P | W | N | W | L | D | F | S | V | T | V | F | E | V | I | I | R | Y | S | P | |
| SCN8A | Q9UQD0 | Domain I, S3 | 193 | P | W | N | W | L | D | F | S | V | I | M | M | A | Y | I | T | E | F | V | N | |
| SCN9A | Q15858 | Domain I, S3 | 187 | P | W | N | W | L | D | F | V | V | I | V | F | A | Y | L | T | E | F | V | N | |
| SCN10A | Q9Y5Y9 | Domain I, S3 | 188 | P | W | N | W | L | D | F | S | V | I | T | L | A | Y | V | G | T | A | I | D | |
| SCN11A | Q9UI33 | Domain I, S3 | 193 | P | W | N | W | L | D | S | I | V | I | G | I | A | I | V | S | Y | I | P | G | |
| CACNA1S | Q13698 | Domain I, S3 | 1181 | P | W | N | V | F | D | F | L | I | V | I | G | S | I | I | D | V | I | L | S | |
| CACNA1C | Q13936 | Domain IV, S3 | 1302 | P | W | N | V | F | D | F | L | I | V | I | G | S | I | I | D | V | I | L | S | |
| TRPC3 | Q13507 | S3 | 468 | L | W | N | V | L | D | F | G | M | L | S | I | F | I | A | A | F | T | A | R | |
| CACNA1A | O00555 | Domain III, S3 | 1312 | L | W | N | I | L | D | F | I | V | V | S | G | A | L | V | A | F | A | F | T | |
| CACNA1E | NP_000712 | Domain III, S3 | 1221 | L | W | N | I | L | D | F | V | V | V | V | G | A | L | V | A | F | A | L | A | |
| CACNA1G | O43497 | Domain III, S3 | 1345 | S | W | N | V | L | D | G | L | L | V | L | I | S | V | I | D | I | L | V | S | |
| CACNA1I | Q9P0X4 | Domain III, S3 | 1245 | S | W | N | V | L | D | G | F | L | V | F | V | S | I | I | D | I | V | V | S | |
| SCN5A | Q14524 | Domain IV, S3 | 1590 | S | W | N | I | F | D | F | V | V | V | I | L | S | I | V | G | T | V | L | | |
| CACNA1A | O00555 | Domain IV, S3 | 1629 | A | W | N | I | F | D | F | V | T | V | L | G | S | I | T | D | I | L | V | T | |
| SCN7A | Q01118 | Domain IV, S3 | 1312 | A | W | N | I | F | D | F | M | V | V | I | F | S | I | T | G | L | C | L | | |
| SCN5A | Q14524 | Domain III, S3 | 1270 | A | W | C | W | L | D | F | L | I | V | D | V | S | L | V | S | L | V | A | N | |
| SCN1A | P35498 | Domain III, S3 | 1283 | A | W | C | W | L | D | F | L | I | V | D | V | S | L | V | S | L | T | A | N | |
| SCN2A | Q99250 | Domain III, S3 | 1273 | A | W | C | W | L | D | F | L | I | V | D | V | S | L | V | S | L | T | A | N | |
| SCN3A | Q9NY46 | Domain III, S3 | 1271 | A | W | C | W | L | D | F | L | I | V | D | V | S | L | V | S | L | V | A | N | |
| SCN4A | P35499 | Domain III, S3 | 1096 | A | W | C | W | L | D | F | L | I | V | D | V | S | I | I | S | L | V | A | N | |
| SCN8A | Q9UQD0 | Domain III, S3 | 1263 | A | W | C | W | L | D | F | L | I | V | A | V | S | L | V | S | L | I | A | N | |
| SCN9A | Q15858 | Domain III, S3 | 1257 | A | W | C | W | L | D | F | L | I | V | D | V | S | L | V | T | L | V | A | N | |
| SCN10A | Q9Y5Y9 | Domain III, S3 | 1217 | A | W | C | W | L | D | F | L | I | V | N | I | S | L | I | S | L | T | A | K | |
| SCN11A | Q9UI33 | Domain III, S3 | 1121 | A | W | C | C | L | D | F | I | I | V | I | V | S | V | T | T | L | I | N | L | |
| KHACN4 | Q9Y3Q4 | S3 | 341 | | W | F | M | V | D | F | I | S | S | I | P | V | D | Y | I | F | L | I | V | E |
| BCNG1 | O60741 | S3 | 220 | | W | F | V | V | D | F | I | S | S | I | P | V | D | Y | I | F | L | I | V | E |
| BCNG2 | Q9UL51 | S3 | 289 | | W | F | V | V | D | F | V | S | S | I | P | V | D | Y | I | F | L | I | V | E |
| Kv10.1 | O95259 | S3 | 295 | | W | F | V | I | D | L | L | S | C | L | P | Y | D | V | I | N | A | F | E | N |
| Kv10.2 | Q8NCM4 | S3 | 292 | | W | F | V | I | D | L | L | S | C | L | P | Y | D | I | I | N | A | F | E | N |
| Kv11.1 | Q12809 | S3 | 496 | | W | F | L | I | D | M | V | A | A | I | P | F | D | L | L | I | F | G | S | G |
| Kv11.2 | Q9H252 | S3 | 345 | | W | F | L | I | D | M | V | A | A | I | P | F | D | L | L | I | F | R | T | G |
| KvH2C | NP_742054 | S3 | 157 | | W | F | L | I | D | M | V | A | A | I | P | F | D | L | L | I | F | G | S | G |
| KvG3-1 | AAH71558 | S3 | 254 | | L | N | I | I | D | L | L | A | I | T | P | Y | Y | I | S | V | L | M | T | V |
| KvSHAL1 | AAH46629 | S3 | 300 | | L | T | L | I | D | L | V | A | I | L | P | Y | Y | I | T | L | L | V | D | G |
| Kv7.1 | P51787 | S3 | 197 | | | I | S | I | D | L | I | V | V | V | A | S | M | V | V | L | C | V | G | S |
| Kv7.4 | P56696 | S3 | 173 | | F | C | V | I | D | F | I | V | F | V | A | S | V | A | V | I | A | A | G | T |
| TRPC1 | P48995 | S3 | 458 | | L | S | F | V | M | N | S | L | Y | L | A | T | F | A | L | K | V | V | A | H |
| TRPM6 | Q9BX84 | S3 | 910 | | W | N | L | T | E | T | V | A | I | G | L | F | S | A | G | F | V | L | R | W |
| PKD2L-2 | Q9NZM6 | S3 | 315 | | I | W | N | W | L | E | L | L | L | L | L | C | F | V | A | V | S | F | N | |

*BLAST alignments of S3 transmembrane segments among homologous proteins demonstrate sequence conservation of positions D1275, V1279, and D1595 in *SCN5A*. Relevant residues and segments of human *SCN5A* are in boldface and bracketed for

easier identification. Colors were randomly selected and assigned to different amino acids to facilitate alignment.