

**Supplemental Digital Content 5. CACNA1S Participant Description Table**

| Characteristics of ClinSeq® Volunteers with CACNA1S Variants |                      |                                   |   |                                       |              |  |
|--|----------------------|-----------------------------------|---|---------------------------------------|--------------|--|
| Nucleotide Change;<br>Predicted Protein Change               | Subject No.          | Race/<br>Ethnicity/Age/<br>Gender | Family History<br>MHS, Heat<br>Illness or<br>Myopathy | Serum CK* <sup>^</sup><br>level (U/L) | LDH<br>(U/L) | Medical/<br>Surgical/ Procedures<br>History  |
| c.900+1G>A Splice-site                                       | 142361 <sup>++</sup> | C/N/63/M                          | Negative  | 84                                    | 131          | Surgeries: appendectomy<br>10 y  |
| c.206C>G;p.Ala69Gly  | N =70                |                                   |   |                                       |              |  |
| c.262A>G;p.Lys88Glu  | 102303               | C/N/45/F                          | Negative  | 78                                    | 125          | Negative   |
| c.502C>T;p.Arg168*   | 197875               | C/N/61/M                          | Negative  | 313                                   | 174          | Colonoscopies x3   |
| c.530C>T;p.Ser177Leu   | 102072               | C/N/60/F                          | Negative  | 139 <sup>^</sup>                      | 149          | Hypothyroidism on THR<br>(TSH 6.31 mIU/mL / FT4<br>1.7 ng/dL)  |
|  | 155322               | C/N/65/F                          | Negative  | 111                                   | 150          | Colonoscopies x2   |
|  | 198019               | C/N/60/F                          | Negative  | 52                                    | 149          | Surgeries: total abdominal<br>hysterectomy, bilateral<br>salpingo-oophorectomy 55<br>y Hypothyroidism on THR |
| c.743C>T;p.Thr248Met   | 146006               | C/N/58/F                          | Negative  | 84*                                   | 180          | Surgeries: total abdominal<br>hysterectomy 52 y<br>Hypothyroidism on THR                                     |
| c.773G>T;p.Gly258Val   | 148573               | C/N/61/M                          | Negative  | 153                                   | 140          | Hypothyroidism on THR  |
|  | 150635               | C/N/45/F                          | Negative  | 48                                    | 159          | Alcohol over consumption:<br>self-reports ≥3 glasses of<br>wine daily  |
| c.773G>A;p.Gly258Asp   | N =14                |                                   |   |                                       |              |  |
| c.862T>G;p.Cys288Gly   | 100395               | C/N/60/F                          | Negative  | 79                                    | 130          | Negative   |
| c.895T>C;p.Tyr299His   | 177243 <sup>+</sup>  | AA/N/58/F                         | Negative  | 247                                   | 223          | Negative   |
|  | 179265 <sup>+</sup>  | AA/N/52/F                         | Negative  | 143                                   | 161          | Negative   |
| c.1112C>T;p.Thr371Met  | 180133               | AA/N/58/F                         | Negative  | 108                                   | 161          | Colonoscopy 56 y   |
| c.1166A>T;p.Asp389Val  | 195059               | C/N/59/F                          | Negative  | 74                                    | 147          | Surgeries: unilateral<br>salpingo-oophorectomy   |
| c.1301T>C;p.Phe434Ser  | 107143               | C/N/62/M                          | Negative  | 264*                                  | 171          | Negative   |
|  | 122640               | C/N/49/F                          | Negative  | 52                                    | 133          | Duane syndrome   |
|  | 115837               | C/N/48/F                          | Negative  | 87*                                   | 121          | Cardiac catheterizations 46<br>y, 48 y   |

**Characteristics of ClinSeq<sup>®</sup> Volunteers with CACNA1S Variants**

| <b>Nucleotide Change;<br/>Predicted Protein Change</b> | <b>Subject No.</b> | <b>Race/<br/>Ethnicity/Age/<br/>Gender</b> | <b>Family History<br/>MHS, Heat<br/>Illness or<br/>Myopathy</b> | <b>Serum CK**<sup>^</sup><br/>level (U/L)</b> | <b>LDH<br/>(U/L)</b> | <b>Medical/<br/>Surgical/ Procedures<br/>History</b>   |
|--|--------------------|--|---|---|----------------------|--|
|  | 182995             | C/N/53/F                                   | Negative  | 63*   | 122                  | Colonoscopy 52 y   |
|  | 130259             | C/N/59/F                                   | Negative  | 99  | 153                  | Negative   |
| c.1313T>G;p.Val438Gly                                  | 126157             | C/N/53/F                                   | Negative  | 90  | 205                  | Surgeries: total abdominal hysterectomy, bilateral salpingo-oophorectomy 50 y<br>Sarcoidosis |
| c.1348G>A;p.Ala450Thr                                  | 186925             | C/N/54/F                                   | Negative  | 65  | 124                  | Surgeries: mitral valve repair 54 y  |
| c.1373T>A;p.Leu458His                                  | N =419             |  |   |   |                      |  |
| c.1493G>A;p.Arg498His                                  | 192035             | C/N/56/M                                   | Negative  | 83  | 174                  | Colonoscopies x2   |
| c.1519G>A;p.Gly507Ser                                  | 190479             | C/N/51/F                                   | Negative  | 114   | 200                  | Hypothyroidism on THR  |
| c.1547C>T;p.Ser516Leu                                  | 150738             | C/N/61/F                                   | Negative  | 56  | 164                  | Negative   |
|  | 179902             | C/N/53/M                                   | Negative  | 68  | 188                  | Marfan Syndrome  |
|  | 154722             | C/N/60/F                                   | Negative  | 113*  | 192                  | Hypothyroidism on THR (TSH 0.15 mIU/mL / FT4 1.7 ng/dL)                                      |
| c.1745G>C;p.Gly582Ala                                  | 100733             | A/N/51/M                                   | Negative  | 217*  | 182                  | Negative   |
| c.1817G>A;p.Ser606Asn                                  | N =16              |  |   |   |                      |  |
| c.1903A>G;p.Met635Val                                  | 105733             | C/N/61/M                                   | Negative  | 70*   | 139                  | Surgeries: cardiac stenting 61 y   |
|  | 184006             | C/N/56/M                                   | Negative  | 186   | 145                  | Negative   |
| c.2047C>T;p.Arg683Cys                                  | 136532             | AA/N/50/M                                  | Negative  | 782*  | 145                  | Hyperuricemia 9.7 mg/dL  |
| c.2440G>A;p.Ala814Thr                                  | 117126+            | C/N/63/M                                   | Negative  | 143   | 126                  | Negative   |
|  | 165903             | C/N/59/F                                   | Negative  | 71*   | 177                  | Negative   |
| c.2467C>T;p.Arg823Trp                                  | 107509             | A/N/52/M                                   | Negative  | 61*   | 115                  | Surgeries: cardiac stenting 52 y   |
| c.2630C>T;p.Ala877Val                                  | 174649             | C/N/56/M                                   | Negative  | 135*  | 166                  | Surgeries: unilateral nephrectomy 40s, cardiac stenting 56 y. Colonoscopy 52 y               |
| c.2957G>A;p.Arg986His                                  | 132619             | C/N/61/F                                   | Negative  | 174 <sup>^</sup>                              | 177                  | Sjogren's syndrome types I & II. Hypothyroidism on THR (TSH 6.53 mIU/mL /                    |

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| <b>Nucleotide Change;<br/>Predicted Protein Change</b> | <b>Subject No.</b> | <b>Race/<br/>Ethnicity/Age/<br/>Gender</b> | <b>Family History<br/>MHS, Heat<br/>Illness or<br/>Myopathy</b> | <b>Serum CK**<sup>^</sup><br/>level (U/L)</b> | <b>LDH<br/>(U/L)</b> | <b>Medical/<br/>Surgical/ Procedures<br/>History</b>                                       |
|--|--------------------|--|---|---|----------------------|--|
|  |                    |  |   |   |                      | FT4 1.5 ng/dL)   |
| c.2992G>A;p.Asp998Asn                                  | 185599             | AA/N/48/F                                  | Negative  | 99  | 114                  | Negative   |
| c.3026C>T;p.Thr1009Met                                 | 131005             | C/N/63/F                                   | Negative  | 168   | 171                  | Negative   |
| c.3190G>A;p.Val1064Ile                                 | 151708             | C/N/55/M                                   | Negative  | 396   | 166                  | Circumferential Pulmonary-Vein Ablation for Chronic Atrial Fibrillation 50s, colonoscopy   |
| c.3364T>C;p.Tyr1122His                                 | 152465             | A/N/63/M                                   | Negative  | 59*   | 138                  | Negative   |
| c.3733A>C;p.Lys1245Gln                                 | 179603             | C/N/58/M                                   | Negative  | 68* <sup>^</sup>                              | 135/ 4.5             | Surgeries: cardiac stenting. TSH =6.39 mIU/mL/ FT4=1.1 ng/dL                               |
| c.3811G>A;p.Ala1271Thr                                 | 197544             | AA/N/47/F                                  | Negative  | 102   | 180                  | Negative   |
| c.3905G>A;p.Arg1302Gln                                 | 142624             | C/N/58/F                                   | Negative  | 80*   | 168                  | Surgeries: knee replacement, total abdominal hysterectomy, bilateral salpingo-oophorectomy |
| c.4060A>T;p.Thr1354Ser                                 | 147218             | C/N/48/M                                   | Negative  | 375* <sup>^</sup>                             | 205                  | TSH =4.3 mIU/mL, FT4=1.3 ng/dL   |
|  | 112126             | C/N/61/F                                   | Negative  | 61  | 143                  | Negative   |
|  | 139980             | C/N/61/M                                   | Negative  | 314*  | 195                  | Negative   |
|  | 117247             | C/N/55/F                                   | Negative  | 59*   | 146                  | Hypothyroidism on THR  |
|  | 104923             | C/N/63/F                                   | Negative  | 100   | 207                  | Negative   |
|  | 141060             | C/N/61/F                                   | Negative  | 93  | 190                  | Negative   |
|  | 195817             | C/N/62/M                                   | Negative  | 132*  | 132                  | Surgeries: cardiac stent at 62 y. Colonoscopies x3   |
|  | 154231             | C/N/58/M                                   | Negative  | 107*  | 161                  | Negative   |
| c.4415G>A;p.Arg1472His                                 | 159259             | C/N/45/M                                   | Negative  | 264   | 175                  | Negative   |
| c.4483_4485del;p.Lys1496del                            | 151463             | A/N/57/M                                   | Negative  | 136 <sup>^</sup>                              | 165                  | Negative   |
| c.4615C>T;p.Arg1539Cys                                 | N =172             |  |   |   |                      |  |
| c.4718C>T;p.Thr1573Met                                 | 184042             | C/N/56/F                                   | Negative  | 51  | 158                  | Negative   |
| c.4731C>G;p.Asp1577Glu                                 | 125959             | C/N/59/F                                   | Negative  | 168*  | 232                  | Hypothyroidism on THR (TSH 0.26 mIU/mL/ FT4 1.7 ng/dL)                                     |

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|--|-------------|-----------------------------------|---|--|--------------|---|
| Nucleotide Change;<br>Predicted Protein Change                           | Subject No. | Race/<br>Ethnicity/Age/<br>Gender | Family History<br>MHS, Heat<br>Illness or<br>Myopathy | Serum CK** <sup>^</sup><br>level (U/L) | LDH<br>(U/L) | Medical/<br>Surgical/ Procedures<br>History   |
| c.4747G>A;p.Glu1583Lys   | 166268      | C/N/60/M                          | Negative  | 86                                     | 175          | Negative  |
|  | 179304      | C/N/45/F                          | Negative  | 36                                     | 134          | Surgeries: total abdominal hysterectomy 44 y  |
|  | 120632      | C/N/59/M                          | Negative  | 48                                     | 114          | Negative  |
| c.4885_4886del; Gln1629Valfs*3   | 114693      | C/N/41/M                          | Negative  | 83                                     | 126          | Surgeries: hip replacement 49 y   |
| c.4954C>T;p.Arg1652Cys   | 101892      | C/N/57/M                          | Negative  | 163 <sup>^</sup>                       | 142          | TSH =7.74 mIU/mL,<br>FT4=1.4 ng/dL  |
| c.4973G>A;p.Arg1658His   | N =116      |                                   |   |  |              |   |
| c.4984A>T;p.Asn1662Tyr   | 152700      | C/N/51/F                          | Negative  | 123*                                   | 216          | Cardiac catheterization 51 y  |
| c.5005G>A;p.Ala1669Thr   | 183652      | C/N/59/F                          | Negative  | 77*                                    | 156          | Surgeries: CABG 51 y,<br>cardiac stents 57.   |
| c.5299C>T;p.Pro1767Ser   | 169812      | C/N/52/M                          | Negative  | 252 <sup>^</sup>                       | 164          | TSH =6.57 mIU/mL,<br>FT4=1.1 ng/dL  |
| c.5399T>C;p.Leu1800Ser   | N =242      |                                   |   |  |              |   |
| c.5510A>C;p.Glu1837Ala   | 138326      | C/HL/46/M                         | Negative  | 164                                    | 131          | Negative  |
|  | 147197      | C/N/50/M                          | Negative  | 123*                                   | 134          | Negative  |
| c.5515C>T;p.Pro1839Ser   | 116697      | C/N/64/M                          | Negative  | 112                                    | 145          | Negative  |
|  | 136180      | C/N/59/F                          | Negative  | 270                                    | 177*         | Surgeries: cardiac stent  |
|  | 139001      | C/N/55/F                          | Negative  | 65*                                    | 129          | Negative  |
|  | 114451      | C/N/57/F                          | Negative  | 50                                     | 72           | Hypothyroidism on THR<br>(TSH 0.36 mIU/mL/ FT4<br>1.3 ng/dL)  |
|  | 115065      | C/N/59/M                          | Negative  | 120                                    | 179          | Negative  |
|  | 184131      | C/N/54/M                          | Negative  | 84*                                    | 176          | Procedures: Endoscopic<br>balloon dilation -esophageal<br>stricture. Gilbert's Disease.<br>TSH =5.31 mIU/mL,<br>FT4=1.1 ng/dL |
|  | 119657      | C/N/52/M                          | Negative  | 118                                    | 177          | Negative  |
| c.5570G>A;p.Ser1857Asn   | 118481      | C/N/62/M                          | Negative  | 182*                                   | 149          | Colonoscopy   |
|  | 158592      | C/N/64/M                          | Negative  | 169*                                   | 166          | Cardiac catheterization   |
|  | 146462      | C/N/57/M                          | Negative  | 114*                                   | 161          | Negative  |
|  |             |                                   |   |  |              |   |

Participant information for *CACNA1S* variants identified in the 870 Clinseq<sup>®</sup> cohort. Medical histories were reviewed for surgeries/ procedures and clinical conditions indicative of myopathy or that elevate serum creatine kinase (CK). Participants were evaluated for: any myopathy, MHS, and/or exertional heat illness; history of elevated CK; hypothyroidism and/or elevated thyroid stimulating hormone (TSH) > 4.00 mIU/mL; hypokalemia <3.3 mmol/L; hyperuricemia > 8.6 mg/dL (uric acid normal range: 3.7-8.6 mg/dL); Statin and Nicotinic Acid use; self-report alcohol use ( $\geq$ 3 bottles/glasses/shots per days); autoimmune conditions: rheumatoid arthritis, psoriasis, lupus, Sjogren's syndrome, scleroderma; and renal disease. Participants race and ethnicity were determined by self report.

Age =age at enrollment. CABG =coronary artery bypass graft. CHCT =caffeine halothane contracture test.

CK =creatinine kinase, range: Female =38-252 U/L; Male =52-386 U/L. Ethnicity: N =Not Hispanic or Latino; HL =Hispanic or Latino. LDH =lactate dehydrogenase, range = 113-226 U/L. K<sup>+</sup> =potassium, range = 3.3-5.1 mmol/L. MHS =Malignant Hyperthermia Susceptible. Nucleotide change =NM\_000540.2. Race: C =Caucasian, A =Asian, AA =African American, O =other, "—" = Not reported. THR =thyroid replacement hormone. TSH range: 0.40–4.00 mIU/mL. Thyroxin, FT4 range =0.9 - 1.5 ng/dL. TURP =transurethral resection of the prostate. "⊕" =participant has an *RYR1* variant. "⊕⊕" =participant with two *RYR1* variants. "★" =participant with another *CACNA1S* variant. "\*" =on Statin. "∧" =elevated serum TSH level. "∨" =low serum TSH level. "---" =sample hemolyzed.