**SUPPLEMENTARY DATA: TABLE OF CONTENT**

**Figure S1: Study flow chart**

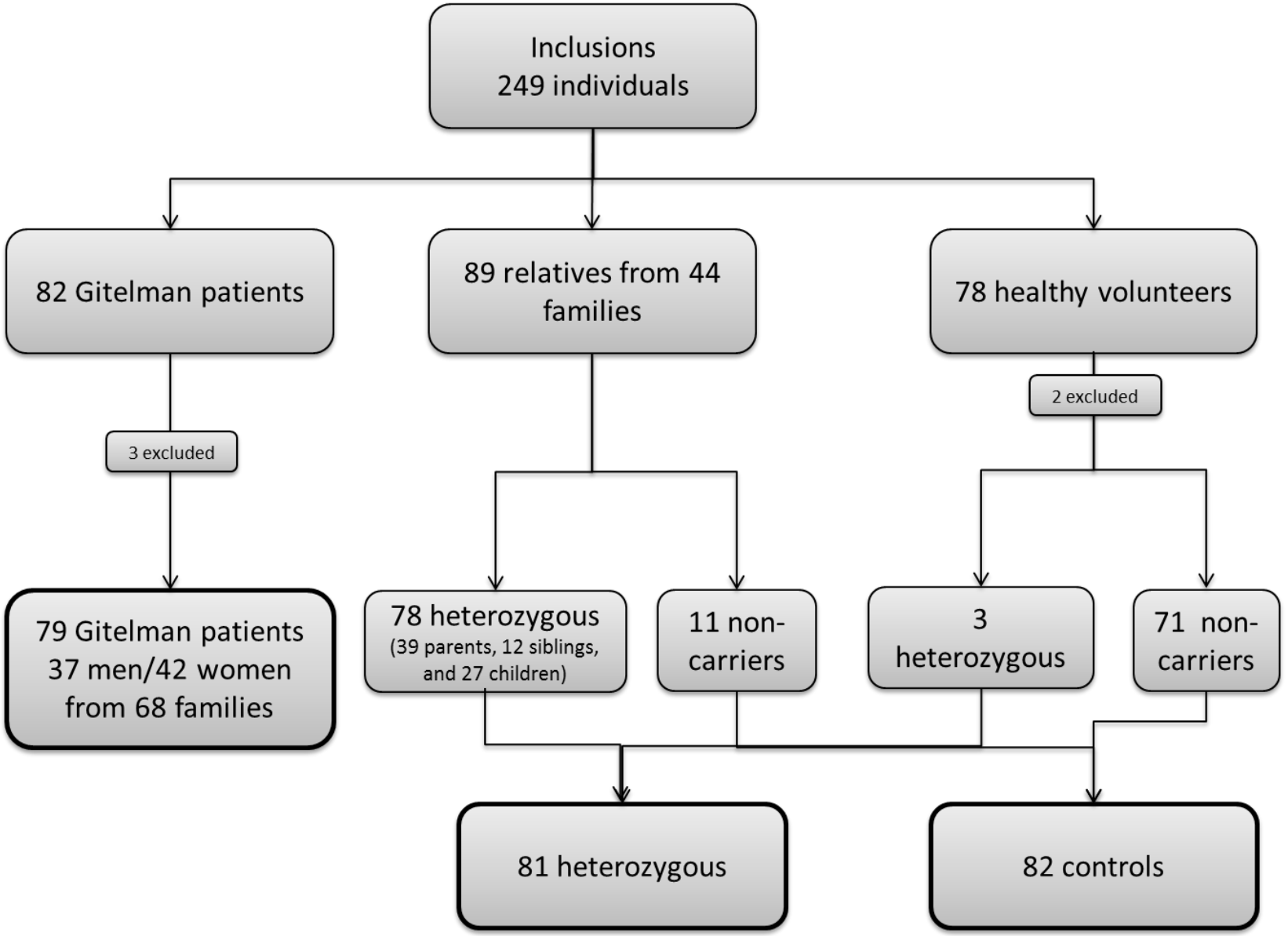
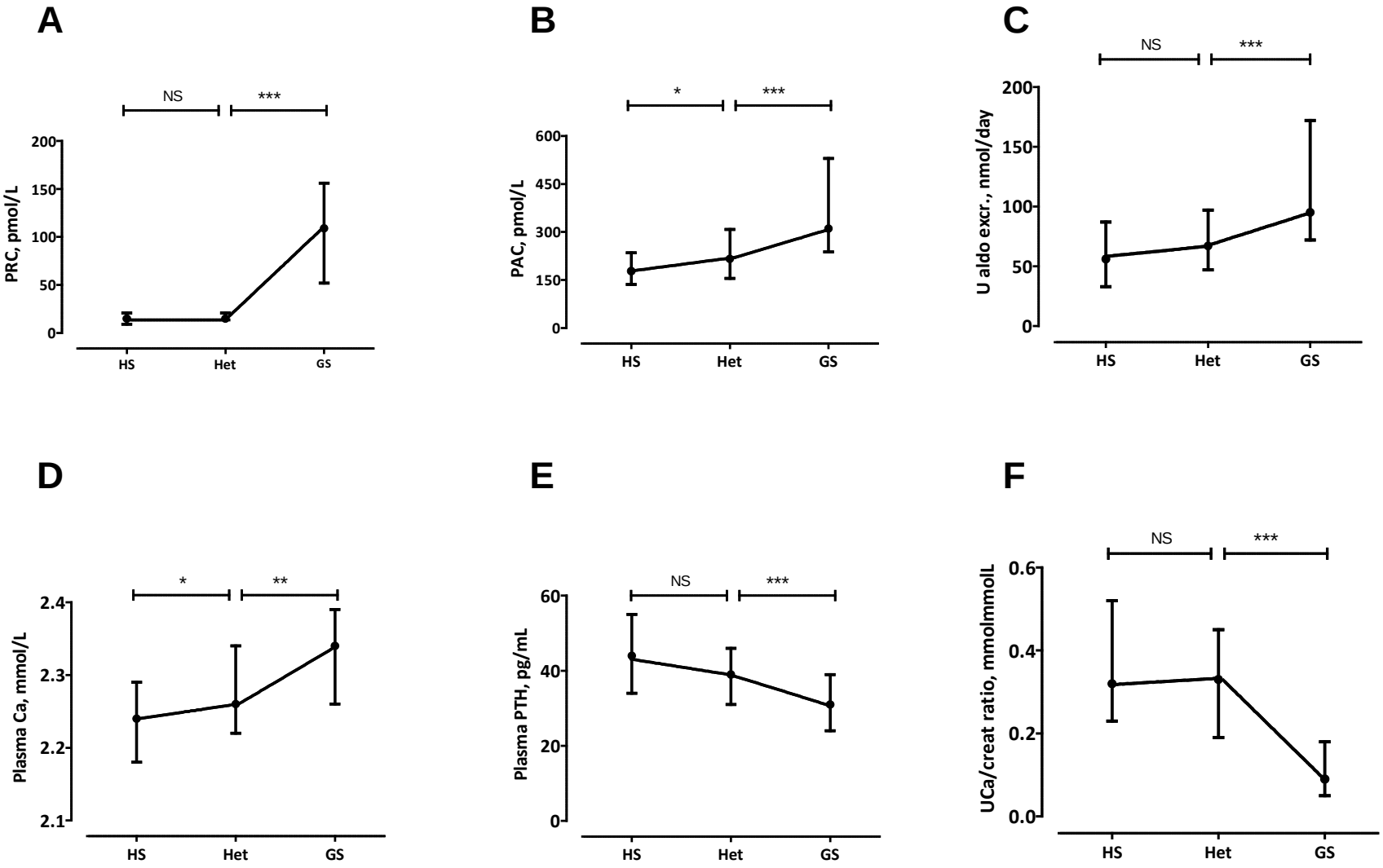


**Figure S2: Evidence for a subtle intermediate phenotype in Het subjects:** Plasma concentrations of renin, aldosterone, calcium, and 7-84 PTH and calcium and aldosterone excretion in HS, Het, and GS groups. Het had significantly higher PAC and plasma calcium than HS and trends for higher aldosterone excretion and plasma PTH concentration. * p> 0.05 ** p< 0.001 ***p<0.0001.



**Supplemental Table 1:** Variants detected in GS patients and Het individuals in the *SLC12A3* gene and their classifications

| Type | Nomenclature cDNA* | Nomenclature protein | Selected classification criteria | ACMG class | Ref | Gitelman Patients | | Heterozygous Carriers |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Homozygous | Compound heterozygous | |
| Large rearrangement | c.(?_-30)_(964+1_965-1) (E1_E7del) | p.? | PVS1, PM2, PM3, PM4, PP1, PP5 | 5 | [1],[2] | | 1 | |
| | c.1169 +773_c.1825+ 247del (E14del)[a] | p.? | PVS1, PM2, PM3, PM4, PP1 | 5 | [2] | | 1 | 1 |
| | (c.2952-1593_*677delins25) (E26del)[a] | p.? | PVS1, PM2, PM3, PM4, PP1 | 5 | [2] | | 2 | 1 |
| | c.(?_-30)_(505+1_506-1)dup (E1_E3dup) | p.? | PVS1, PM2, PM3, PM4, PP1 | 5 | [2] | | 1 | |
| | c.(?_-30)_(852+1_853-1)dup (E1_E6dup) | p.? | PVS1, PM2, PM3, PM4 | 5 | This study | | 1 | |
| Frameshift | c.20_21del | p.Thr7Argfs*22 | PVS1, PM2, PM4 | 5 | [2] | 0 | 2 | |
| | c.56_57dup | p.Phe20Alafs*8 | PVS1, PM2, PP1 | 5 | This study | 1 | 0 | 3 |
| | c.293_296dup | p.His99Glnfs*9 | PVS1, PM2, PM3, PM4 | 5 | [2] | | 1 | |
| | c.971_984del | p.Phe325Glyfs*3 | PVS1, PM2, PM4 | 5 | [2] | | 1 | |
| | c.1196_1202dup | p.Ser402* | PVS1, PS4, PM3, PM4, PP5 | 5 | [3] | | 3 | |
| | c.1796_1797dup | p.Leu600Serfs*12 | PVS1, PM2, PM3, PM4, | 5 | [2] | | 1 | 1 |
| | c.1805_1806del | p.Tyr602Cysfs*31 | PVS1, PM2, PM3, PM4, PP5 | 5 | [4] | | 1 | |
| | c.1832del | p.Asn611Ilefs*61 | PVS1, PM2, PM3, PM4, | 5 | [2] | | 2 | 1 |
| | c.2089_2095del | p.Thr697Glyfs*2 | PVS1, PS4, PM2, PM3, PM4 | 5 | [5] | | 1 | |
| | c.2301del | p.Phe767Leufs*8 | PVS1, PM2, PM3, PM4 | 5 | [2] | | | 1 |
| | c.2379dup | p.Phe794Valfs*2 | PVS1, PM2, PM3, PM4 | 5 | [6] | | 1 | 2 |
| | c.2877_2878del | p.Arg959Serfs*11 | PVS1, PM2, PM3, PM4, PP5 | 5 | [7] | | 1 | |
| | c.2961dup | p.Ile988Hisfs*60 | PVS1, PM1, PM2, PM3, PM4, PP5 | 5 | [8] | | 1 | 1 |
| Nonsense | c.1687C>T | p.Gln563* | PVS1, PM2, PM3, PM4, PP1 | 5 | This study | | 1 | 1 |
| | c.3052C>T | p.Arg1018* | PVS1, PM1, PM2, PM3, PM4 | 5 | [9] | | 3 | 2 |
| Splice | c.429+2dupT | p.? | PVS1, PM1, PM2, PM3, PP3 | 5 | This study | | 1 | |
| | c.429+1_c.429+18del | p.? | PVS1, PM1, PM2, PM3, PP3 | 5 | This study | | 1 | |
| | c.852+1G>A | p.? | PVS1, PM2, PM3, PP1, PP3 | 5 | This study | | 2 | 3 |
| | c.1095+4A>G | p.? | PM2, PM3, PP1, PP3 | 4 | [2] | | 1 | |
| | c.1180+1G>T | p.? | PVS1, PS4, PM1, PM3, PP3, PP5 | 5 | [10] | 1 | 3 | 1 |
| | c.1444-10G>A | p.? | PM2, PM3, PP1, PP3 | 4 | This study | | | 1 |
| | c.1826-1G>A | p.? | PVS1, PM1, PM2, PM3, PP3 | 5 | [2] | | 1 | 1 |
| | c.1925+1 G>A | p.? | PVS1, PM1, PM2, PM3, PP3, PP5 | 5 | [6] | | 1 | 1 |
| | c.2548+1G>T | p.? | PVS1, PM1, PM2, PM3, PP3, PP5 | 5 | [6] | | 1 | |
| | c.2660+1G>A | p.? | PVS1, PS4, PM1, PM2, PM3, PP3, PP5 | 5 | [11] | 1 | | |
| | c.2883+1G>T | p.? | PVS1, PS4, PM1, PM2, PM3, PP3, PP5 | 5 | [12] | 1 | 6 | 3 |
| In frame | c.674_697delins9 | p.Phe225_Met233delinsLeuHisThrVal | PM1, PM2, PM3 | 4 | [2] | 0 | 1 | 1 |

| Type | Nomenclature cDNA* | Nomenclature protein | Selected classification criteria | ACMG class | Ref | Gitelman Patients | | Heterozygous Carriers |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Homozygous | Compound heterozygous | |
| Missense | c.247C>T | p.Arg83Trp | PS3,PS4, PM2, PM5, PP3, PP5 | 5 | [2], [13] | | 1 | |
| | c.472C>T | p.Arg158Trp | PM2, PM3, PM5, PP1, PP3 | 4 | This study | | 1 | |
| | c.514T>C | p.Trp172Arg | PS4, PM2, PM3, PP3, PP5 | 5 | [5] | | | 1 |
| | c.533C>T | p.Ser178Leu | PM1, PM2, PM3, PP1, PP3, PP5 | 5 | [14] | | 1 | |
| | c.625C>T | p.Arg209Trp | PS3, PM2, PM5, PP3, PP5 | 5 | [12],[15] | 1 | | |
| | c.644T>C | p.Leu215Pro | PS3, PS4, PM2, PP3, PP5 | 5 | [16],[17] | 1 | | |
| | c.689G>A | p.Gly230Asp | PM1, PM2, PM3, PP1, PP5 | 4 | [11] | | 1 | |
| | c.791G>C | p.Gly264Ala | PS3, PM5, PM3, PP5 | 4 | [18],[19] | | | 3 |
| | c.910A>C | p.Thr304Pro | PM2, PM3, PM5, PP5 | 5 | [20] | | 1 | |
| | c.911C>T | p.Thr304Met | PS4, PM1, PM2, PM5, PP3 | 4 | [2] | | 1 | 1 |
| | c.938C>T | p.Ala313Val | PS4, PM2, PM3, PP5 | 4 | [14] | | 4 | 3 |
| | c.947G>T | p.Gly316Val | PS3, PS4, PM2, PM3, PM5, PP3 | 5 | [5],[21] | | 1 | 1 |
| | c.1085G>T | p.Gly362Val | PM1, PM2, PM3, PP3 | 4 | [2] | | 1 | |
| | c.1145C>T | p.Thr382Met | PS4, PM3, PM2, PP1, PP3, PP5 | 5 | [6] | | 1 | |
| | c.1195C>T | p.Arg399Cys | PS3, PS4, PM2, PM3, PM5, PP3 | 5 | [14], [21] | | 2 | 2 |
| | c.1315G>A | p.Gly439Ser | PS3, PM2, PM3, PP3, PP5 | 5 | [3],[17] | | 2 | 1 |
| | c.1387G>A | p.Gly463Arg | PS3, PS4, PM1, PM2, PM5, PP3 | 5 | [2],[13] | | 2 | |
| | c.1424C>G | p.Ser475Cys | PS3, PS4, PM2, PP3, PP5 | 5 | [2],[22] | | 2 | |
| | c.1432A>G | p.Lys478Glu | PM1, PM2, PP3, PP5 | 4 | [3] | | 2 | |
| | c.1519C>T | p.Arg507Cys | PS4, PM2, PM3, PP3 | 4 | [2] | | 1 | |
| | c.1567G>A [b] | p.Ala523Thr/p.Cys482_Ala523Leufs*6 | PS3, PM2, PM3, PP3, PP5 | 5 | [21] | | 2 | 1 |
| | c.1664C>T | p.Ser555Leu | PS3, PS4, PM1, PM2, PP3, PP5 | 5 | [14],[21] | | 2 | 1 |
| | c.1763C>T | p.Ala588Val | PS3, PM3, PM2, PP3, PP5 | 5 | [12],[21] | | 2 | |
| | c.1844C>T | p.Ser615Leu | PM1, PM2, PM5, PP3, PP5 | 4 | [14] | | 1 | |
| | c.1928C>T | p.Pro643Leu | PS4, PM1, PM2, PM3, PP3, PP5 | 5 | [14] | | 2 | 2 |
| | c.1946C>T | p.Thr649Met | PM1, PM2, PM3, PM5, PP3, PP5 | 4 | [6] | | 2 | |
| | c.1963C>T | p.Arg655Cys | PM1, PM5, PM2, PM3, PP3, PP5 | 4 | [16] | | 2 | |
| | c.1964G>A | p.Arg655His | PS4, PM1, PM2, PM3, PM5, PP3, PP5 | 5 | [12] | | 1 | 1 |
| | c.1967C>T | p.Pro656Leu | PM1, PM2, PM3, PP5 | 4 | [2] | | 2 | 1 |
| | c.2191G>A | p.Gly731Arg | PS3, PS4, PM1, PM2, PM3, PP3, PP5 | 5 | [3],[13] | | 5 | 2 |
| | c.2213T>G | p.Leu738Arg | PS3, PM2, PM3, PP3, PP5 | 5 | [16],[15] | | 1 | 2 |
| | c.2221G>A | p.Gly741Arg | PS3, PS4, PM1, PM2, PM3, PP3, PP5 | 5 | [12],[15],[17] | | 9 | 7 |
| | c.2576T>C | p.Leu859Pro | PS3, PS4, PM2, PM3, PP3, PP5 | 5 | [12], [13] | 1 | 16 | 6 |
| | c.2581C>T | p.Arg861Cys | PS4, PM1, PM3, PM5 , PP3, PP5 | 5 | [16] | | 9 | 9 |
| | c.2687G>A | p.Arg896Gln | PM1, PM2, PM3, PP3, PP5 | 4 | [23] | 1 | 2 | 4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | c.2690T>C | p.Leu897Pro | PM1, PM2, PM3, PP3 | 4 | [2] | | 1 | |
| | c.2827C>T | p.Arg943Trp | PS4, PM2,PM3, PP3 | 5 | [2] | | 1 | 3 |
| | c.2890C>T | p.Arg964Trp | PM1, PM2, PM3, PM5 | 4 | [2] | | 1 | |
| | c.2981G>A | p.Cys994Tyr | PS3, PS4, PM1, PM2, PM3, PP3, PP5 | 5 | [5],[17] | | 11 | 4 |
| | c.2993T>G | p.Leu998Arg | PM1, PM2, PM3, PP3 | 4 | This study | | 1 | |
| | c.3006G>C | p.Trp1002Cys | PM1, PM2, PM3, PP3 | 4 | [2] | | 1 | 1 |
| | c.3077C>T | p.Thr1026Ile | PS3, PM1, PM2, PP3, PP5 | 5 | [15] | 1 | | |

*Numbering is according to the cDNA sequence (GenBank: NM_000339.2). The A of the ATG of the initiator methionine codon is nucleotide 1. Novel mutations are in red. [a] The breakpoints of these two large deletions were characterized. [b]Last nucleotide of exon 12 with new transcript lacking exon 12. PVS: very strong evidence of pathogenicity. PVS1: null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function is a known mechanism of disease. PS: strong evidence of pathogenicity. PS1: same amino acid change as a previously established pathogenic variant regardless of nucleotide change. PS3: well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product. PS4: the prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls. PM: moderate evidence of pathogenicity. PM1: Located in a mutational hot spot and/or critical and well-established functional domain without benign variation. PM2: absent from controls (or at extremely low frequency if recessive) in gnomAD database. PM3: detected in *trans* with a pathogenic variant (the phase was determined). PM4: protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants. PM5: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before. PP: supporting evidence of pathogenicity. PP1: co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease. PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product. PP5: Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation.

**Supplementary Table 2:** PTH, vitamin D metabolites, and bone remodeling markers

| | **HS** (n=82) | **Het** (n=81) | **GS** (n=77) | **ANOVA** ***P* value** | **Unpaired t-test HS vs. Het** |
|---|---|---|---|---|---|
| **Plasma** | | | | | |
| Calcium, mmol/L | 2.24 [2.18;2.29] *** | 2.26 [2.22;2.35] ** | 2.34 [2.26;2.39] | *<0.0001* | *0.0104* |
| PTH 7-84, pg/mL | 44 [34;55] *** | 39 [31;46] *** | 31 [24;39] | *<0.0001* | *0.111* |
| 25OH vitamin D, nmol/l | 58 [38;70] ** | 61 [42;76] | 64 [44;87] | *0.0119* | 0.569 |
| Calcitriol , pmol/l | 137 [106;158] * | 141 [114;176] *** | 116 [91;140] | *<0.0002* | 0.369 |
| Osteocalcin, ng/ml | 24 [20;30] *** | 24 [20;30] *** | 17 [13;22] | *<0.0001* | 1.0000 |
| Alkaline phosphatase, UI/l | 55 [44;67] | 56 [47;63] | 51 [42;61] | *0.101* | 0.989 |
| Collagen 1 C tel., nmol/l | 3.45 [2.60;4.50] *** | 3.00 [2.10;4.15] *** | 1.70 [1.20;2.80] | *<0.0001* | 0.641 |

HS: healthy subjects; Het: heterozygous carrier; GS: Gitelman syndrome. Difference between groups was first evaluated with ANOVA. If significant, Tukey test was performed to compare HS and Het and Dunn's test was performed to compare HS vs. GS and Het vs. GS (* P<0.05, ** P<0.001 *** P<0.0001). Data are medians [IQR].

**Supplemental Table 3 :** A posteriori calculation of the sample size required to yield 80% power

| Parameter | HS (n=82)[a] | Het (n=74)[a] | Actual p value | Sample size required[b] |
|---|---|---|---|---|
| SBP, mmHg | 117 [111;125] | 114 [106;127] | 0.442 | 589 |
| DBP, mmHg | 71 [65;76] | 68 [63;75] | 0.335 | 1,313 |
| Heart rate, bpm | 69 [62;76] | 69 [62;76] | 0.996 | 118,174 |
| Sodium, mmol/L | 140 [139.141] | 140 [139;142] | 1.000 | - |
| Potassium, mmol/L | 4.0 [3.8;4.2] | 3.9 [3.7;4.1] | 1.000 | 172 |
| Calcium, mmol/l | 2.24 [2.18;2.29] | 2.26 [2.21;2.34] | 0.0190 | 97 |
| Magnesium, mmol/l | 0.80 [0.75;0.83] | 0.80 [0.76;0.84] | 1.000 | 790 |
| PTH7-84, pg/mL | 44 [34;55] | 39 [30;40] | 0.086 | 158 |
| PRC, mUI/ml | 15 [9;21] | 16 [11;23] | 0.674 | 844 |
| PAC, pmol/L | 178 [137. 230] | 213 [154;305] | 0.081 | 151 |
| Na excr, mmol/24h | 147 [99;172] | 133 [93;189] | 0.930 | 54,906 |
| Mg excr, mmol/24h | 3.35 [2.48;4.21] | 3.32 [2.68;4.26] | 0.951 | 22,112 |
| Ca excr, mmol/24h | 3.76 [2.62;5.54] | 3.51 [2.24;5.23] | 1.000 | 600 |
| UAE, nmol/24h | 56 [33;87] | 67 [48;95] | 0.109 | 182 |

[a]Actual median [IQR] of the parameters measured in HS and Het and actual mean difference observed between group. Considering these differences, the two-sided type 1 error rate is 0.017%. [b]The sample size required per group was calculated to yield 80% power (likelihood that a study will detect the actual difference effect when this difference is indeed present).

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