# SUPPLEMENTARY DATA

**Table S1:** Reverse Transcription-PCR (RT-PCR) Conditions

| Gene | Exons | Amplicon size (bp) | Annealing Temperature (°C) | Mg2+ (mM) | Forward PCR Primer (5'-3') | Reverse PCR Primer (5'-3') |
|---|---|---|---|---|---|---|
| SMAD3 | 6 to 9 | 688 | 61 | 1.5 | TAGGGCTGCTCTCCAATGTCAA | TGGGGCCAAAGGGTAAATGTGT |
| SMAD4 | 8 to 11 | 519 | 56 | 1.5 | TATAAATGAGGCTGGTCTAGGG | GACATGCCTACTACGACCGTAG |

**Table S2a:** Sample characteristics of nucleotide diversity studies

| | Base Pairs Screened (bp) | | | | |
|---|---|---|---|---|---|
| **Site of Variation** | **This Study (SMAD3)** | **This Study (SMAD4)** | **Cargill et al** | **Halushka et al** | **Ten Ashbroek et al** |
| **Non-Coding** | 1135 | 1090 | 60,410 | 102607 * | 8884 ** |
| **Coding** | 415 | 688 | 138,823 | 86,946 | 42,270 |
| Total | 1550 | 1778 | 196,233 | 189,553 | 51,154 |
| Characteristic of Study Genes | Signaling | Signaling | cardiovascualr, endocrine, neurological systems genes | Blood-Pressure genes | Highly Conserved essential genes |
| **Total # Individuals Screened** | 408 Cases 710 Contr | 408 Cases 710 Contr | 57 (20 european, 14 asian, 10 African american, 7 african pygmies, 10 european) | 74 (40 zimbawe, 32 Michigan, 3 N. European) | 36 (4 chinese, 4 japanese, 2 oterh asians, 1 india, 1 saudi arabia, 4 african american, 4 hispanic american, 5 white of south european origin, 11 whites of central/eur origin) |

* Including 5' and 3' UTR
** 3' UTR analyzed

**Table S2b:** Polymorphism counts for nucleotide diversity studies

| | # Polymorphisms detected | | | | |
|---|---|---|---|---|---|
| | This Study (SMAD3) | This Study (SMAD4) | Cargill et al | Halushka et al | Ten Ashbroek et al |
| **Non-Coding** | 10 (Control) 4 (Case) | 10 (Control) 6 (Case) | 168 | 487 * | N/A |
| **Coding** | 0 (Control) 0 (Case) | 2 (Control) 2 (Case) | 392 | 387 | 65 |
| Synonymous | 0 (Control) 0 (Case) | 1 (Control) 1 (Case) | 207 | 178 | N/A |
| Non-synonymous | 0 (Control) 0 (Case) | 1 (Control) 1 (Case) | 185 | 209 | N/A |
| **Total** | 10 (Control) 4 (Case) | 12 (Control) 8 (Case) | 560 | 874 | 65 |

* 150 variants were intronic, the remaining were found in the 3' UTR

## Table S3: Detailed Bioinformatic output for biologically relevant variants

| SMAD3 (MH2) | Genetic Variant | Case or Control | ASSA (WT > Mut) | Predicted Outcome |
|---|---|---|---|---|
| **Exon 9** | IVS8-55 A>G | Control | Branch Point Change 2.6 > -9.3 (-453.8%) | Abolished branch point |

| SMAD4(MH2) | Genetic Variant | Case or Control | ASSA (WT > Mut) | Predicted Outcome |
|---|---|---|---|---|
| **Exon 10** | IVS10+109 A>G | Cases and Control | Cryptic donor -9.1 > 3.7 (+345.9%) | Creation of a cryptic donor site 108 bp upstream of wildtype donor |
| **Exon 11** | IVS11+126 delTATATTA | Control | Cryptic branch point -7.7 > 3.8 (+302.6%) | Creation of a cryptic branch site 113 bp upstream of wildtype donor |
| **Exon 12** | IVS12-52 A>T | Control | Cryptic donor -4.5 > 3.3 (+236%) | Creation of a cryptic donor site 53 bp upstream of wildtype donor site |
| | IVS12-33 T>A | Case | Cryptic branch point -4 > 7.9 (+297.5%) | Creation of a cryptic branch site 32 bp upstream of wildtype acceptor |

| SMAD4(MH2) | Genetic Variant | Case or Control | Evolutionary Conservation | | FASTsnp | |
|---|---|---|---|---|---|---|
| | | | **SIFT** | **PolyPhen** | **ESE-Finder** | **Predicted Outcome** |
| **Exon 10** | c.1214T>C / p.Phe362Phe | Cases and Control | NA | NA | Loss SC35 & Srp55 | Loss of ESE Motifs |
| **Exon 12** | c.1478G>A / p.Asp450Asp | Case | NA | NA | Gain Srp55: 3.3435 Loss SF2/ASF and SC35 | Loss/Gain of ESE Motifs |
| **Exon 13** | c.1701A>G / p.Ile525Val | Control | Tolerated | Benign | None Found | No Changes to Splicing Pattern |

**Table S4a**: Detailed real-time PCR results for SMAD3 groups

| Patient ID | Age | Familial | SMAD3 Variant(s) | SMAD3/B2M Ratio | Classification | Mean ± SD |
|---|---|---|---|---|---|---|
| P3 | 39 | OFBCR | | 0.36 ± 0.02 | BC-REF | |
| P6 | 51 | OFBCR | | 0.64 ± 0.01 | BC-REF | |
| P7 | 47 | | | 1.94 ± 0.1 | BC-REF | 3.83±0.78 |
| P2 | 57 | | | 3.69 ± 0.1 | BC-REF | |
| P5 | 44 | | | 12.52± 1.8 | BC-REF | |
| P1 | 53 | OFBCR | IVS8+23A>C | 1.11 ± 0.08 | BC-VAR | |
| P4 | 45 | OFBCR/FDR | IVS9+132 A>T | 2.39 ± 0.1 | BC-VAR | 3.19±0.35 |
| P8 | N/A | OFBCR/FDR | IVS8+23A>C | 6.08 ± 0.7 | BC-VAR | |
| C13 | 47 | | | 0.15±0.002 | CO-REF | |
| C7 | 43 | | | 0.43±0.03 | CO-REF | |
| C5 | 49 | | | 0.47±0..03 | CO-REF | |
| C24 | 37 | | | 0.48±0.02 | CO-REF | |
| C28 | 62 | | | 0.49±0.03 | CO-REF | |
| C23 | 49 | | | 0.5±0.07 | CO-REF | 0.93±0.066 |
| C1 | 44 | | | 0.83±0.1 | CO-REF | |
| C4 | 37 | | | 0.84±0.05 | CO-REF | |
| C15 | 31 | OFBCR/FDR | | 1.61±0.05 | CO-REF | |
| C27 | 60 | | | 2.05±0.2 | CO-REF | |
| C2 | 44 | | | 2.98±0.2 | CO-REF | |
| C22 | 67 | | IVS8+48T>G | 0.35±0.05 | CO-VAR | |
| C14 | 50 | | IVS8+161C>T | 0.37±0.008 | CO-VAR | |
| C10 | 45 | | IVS6-132C>T | 0.42±0.03 | CO-VAR | |
| C9 | 54 | | IVS8+23A>C | 0.59±0.05 | CO-VAR | |
| C25 | 52 | | IVS7+69G>C, IVS8-55 A>G | 0.60±0.2 | CO-VAR | |
| C18 | 46 | | IVS8+48T>G | 0.62±0.1 | CO-VAR | 2.03±0.071 |
| C26 | 66 | | IVS7+69G>C | 0.7±0.07 | CO-VAR | |
| C3 | 42 | | IVS8+23A>C | 0.73±0.07 | CO-VAR | |
| C6 | 34 | | IVS8-211 C>T | 0.91±0.05 | CO-VAR | |
| C19 | 43 | | IVS7+69G>C, IVS8-55 A>G | 1.68±0.1 | CO-VAR | |
| C8 | 43 | OFBCR/FDR | IVS8+23A>C | 1.82±0.2 | CO-VAR | |
| C17 | 48 | | IVS8+23A>C | 1.92±0.2 | CO-VAR | |
| C11 | 50 | | IVS7+69G>C, IVS8-55 A>G | N/A | Removed | |
| C20 | 46 | | | N/A | Removed | N/A |
| C21 | 45 | | IVS8-170C>T | N/A | Removed | |
| C12 | 52 | | | ND | ND | |
| C16 | 35 | | IVS8+23A>C | ND | ND | N/A |
| P9 | N/A | OFBCR | | ND | ND | |

Removed: Samples removed due to low mRNA quality
ND: Not done due to limitation in template cDNA

**Table S4b**: Detailed real-time PCR results for SMAD4 groups

| Patient ID | Age | Familial | SMAD4 Variant(s) | SMAD4/B2M Ratio | Classification | Mean ± SD |
|---|---|---|---|---|---|---|
| P4 | 45 | OFBCR/FDR | | 0.12±0.02 | BC-REF | |
| P8 | N/A | OFBCR/FDR | | 1.8±0.3 | BC-REF | 0.72±0.17 |
| P1 | 53 | OFBCR | | 0.23±0.01 | BC-REF | |
| P7 | 47 | | IVS10 +132 delA | 0.84±0.03 | BC-VAR | |
| P6 | 51 | OFBCR | IVS10-33 T>A | 0.91±0.09 | BC-VAR | |
| P3 | 39 | OFBCR | IVS8+109 A>G | 0.97±0.01 | BC-VAR | 1.96±0.42 |
| P5 | 44 | | IVS9+118 A>G | 2.02±0.2 | BC-VAR | |
| P2 | 57 | | IVS10+41 G>A | 2.08±0.5 | BC-VAR | |
| P9 | N/A | OFBCR | c.1478G>A / p.Asp450Asp | 4.96±1.1 | BC-VAR | |
| C22 | 67 | | | 0.14±0.01 | CO-REF | |
| C3 | 42 | | | 0.67±0.02 | CO-REF | |
| C18 | 46 | | | 0.68±0.06 | CO-REF | |
| C17 | 48 | | | 0.72±0.01 | CO-REF | |
| C19 | 43 | | | 0.77±0.1 | CO-REF | |
| C9 | 54 | | | 0.79±0.04 | CO-REF | 1.08±0.1 |
| C25 | 52 | | | 0.8±0.05 | CO-REF | |
| C16 | 35 | | | 1.1±0.09 | CO-REF | |
| C8 | 43 | OFBCR/FDR | | 1.12±0.1 | CO-REF | |
| C6 | 34 | | | 1.19±0.2 | CO-REF | |
| C10 | 45 | | | 1.58±0.1 | CO-REF | |
| C28 | 62 | | IVS10 +132 delA | 0.59±0.06 | CO-VAR | |
| C23 | 49 | | IVS8-121 A>C | 0.67±0.1 | CO-VAR | |
| C7 | 43 | | IVS10-62 A>T | 0.69±0.06 | CO-VAR | |
| C12 | 52 | | c.1214T>C / p.Phe362Phe | 0.77±0.1 | CO-VAR | |
| C24 | 37 | | c.1701A>G / p.Ile525Val | 0.78±0.09 | CO-VAR | |
| C14 | 50 | | IVS8+109 A>G | 0.8±0.08 | CO-VAR | |
| C13 | 47 | | IVS7-121 A>C | 0.85±0.08 | CO-VAR | 0.85±0.048 |
| C1 | 44 | | IVS8+44 T>C | 0.87±0.2 | CO-VAR | |
| C5 | 49 | | IVS10 +132 delA | 0.92±0.07 | CO-VAR | |
| C15 | 31 | OFBCR/FDR | IVS8+109 A>G | 0.93±0.1 | CO-VAR | |
| C2 | 44 | | IVS9+68 delGAA | 1.06±0.09 | CO-VAR | |
| C27 | 60 | | IVS10 +132 delA | 1.07±0.05 | CO-VAR | |
| C4 | 37 | | IVS8+109 A>G | 1.09±0.2 | CO-VAR | |
| C11 | 50 | | | N/A | Removed | |
| C20 | 46 | | IVS11+53 A>G | N/A | Removed | N/A |
| C21 | 45 | | | N/A | Removed | |
| C26 | 66 | | | ND | ND | N/A |

Removed: Samples removed due to low mRNA quality
ND: Not done due to limitation in template cDNA