**Two locus inheritance of non-syndromic midline craniosynostosis via rare *SMAD6* and common *BMP2* alleles**

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SUPPLEMENTARY FILE 1

**Supplementary File 1A.**

**Exome Sequencing Quality Statistics for all members of craniosynostosis kindreds (n=455) and autism controls (n=3,337).**

|  |  |  |
| --- | --- | --- |
|  | Cases | Controls |
| Read length (bp) | 74 | 74 |
| Number of reads per sample (M) | 81.30 | 115.29 |
| Percentage of targeted bases with more than 8 independent reads (%) | 94.68 | 94.88 |
| Percentage of targeted bases with more than 20 independent reads (%) | 83.76 | 86.70 |

**Supplementary File 1B.**

**TDT of an intergenic *BMP2* risk allele in *SMAD6* mutation carriers with craniosynostosis.**

|  |  |  |
| --- | --- | --- |
|  | Transmitted | Non-transmitted |
| Craniosynostosis (+) | 11 | 2 |
| Expectation | 6.5 | 6.5 |

P=0.013 via Chi square with 1 Df.

Transmission disequilibrium test of *BMP2* risk locus rs1884302 allele ‘C’ in 13 kindreds with rare, damaging *SMAD6* variants.

**TDT of an intronic *BBS9* risk allele in *SMAD6* mutation carriers with craniosynostosis.**

|  |  |  |
| --- | --- | --- |
|  | Transmitted | Non-transmitted |
| Craniosynostosis (+) | 4 | 10 |
| Expectation | 7 | 7 |

P=0.89 via Chi square with 1 Df.

Transmission disequilibrium test of *BBS9* risk locus rs10262453 allele ‘A’ in 13 kindreds with rare, damaging *SMAD6* variants.

**Supplementary File 1C.**

**Optimized two locus and single locus parametric models of genotype specific penetrances for *SMAD6* and *BMP2***

|  |  |  |
| --- | --- | --- |
| **Genotype**  **(*SMAD6*; *BMP2*)** | **Penetrance in**  **two locus model (*SMAD6*/*BMP2*)** | **Penetrance in**  **single locus model**  **(*SMAD6*)** |
| +,D; C,C | 1 | 0.2 |
| +,D; C,T | 1 | 0.2 |
| +,D; T,T | 0.09 | 0.2 |
| +,+; C,C | 0.0032 | 0.0032 |
| +,+; C,T | 0.0008 | 0.0008 |
| +,+; T,T | 0.0002 | 0.0002 |

Alleles at *SMAD6* (+ or D) are wild-type or have rare damaging variants, respectively. For alleles of a common SNP near *BMP2*, rs1884302, which is associated with midline craniosynostosis15, allele C confers increased risk. Penetrance estimates under the maximum lod score models are shown. Both models specify a phenocopy rate of 0.0002 (based on a prevalence of 1/4000 for nonsyndromic midline craniosynostosis, with 7% of these cases attributable to *SMAD6* + *BMP2* risk alleles as well as a 4- fold increase in risk conferred by each *BMP2* risk allele based on the previous GWAS15.

**Supplementary File 1D.**

**Family specific lod scores for each kindred under the two locus and single locus models.**

|  |  |  |
| --- | --- | --- |
| **Kindred** | **Lod score, two locus model** | **Lod score, single locus model** |
| Q78fs\*41 | .564 | .300 |
| R345fs\*194 | .220 | .645 |
| A353fs\*187 | 1.01 | .548 |
| R281fs\*13 | 0.564 | 0.300 |
| T306A | 0.682 | 0.295 |
| E374\* | NA | NA |
| S130fs\*146 | 0.300 | 0.301 |
| Q223\* | 0.662 | 0.249 |
| P323L | 0.564 | 0.300 |
| G390C | NA | NA |
| E407\* | 1.13 | 0.600 |
| R465C | 0.743 | 0.344 |
| I490T | 0.938 | 0.340 |
| **Total lod score** | **7.37** | **4.22** |
| **Odds ratio in favor of linkage** | **2.3 x 107:1** | **1.7 x 104:1** |

Lod scores under the parametric models specified in Supplementary File 1C are shown for each kindred harboring a *SMAD6* variant. Kindreds with *de novo* mutation in the proband are not informative for segregation (NA). The two locus model is >1,400x more likely than the single locus model under the optimized models.

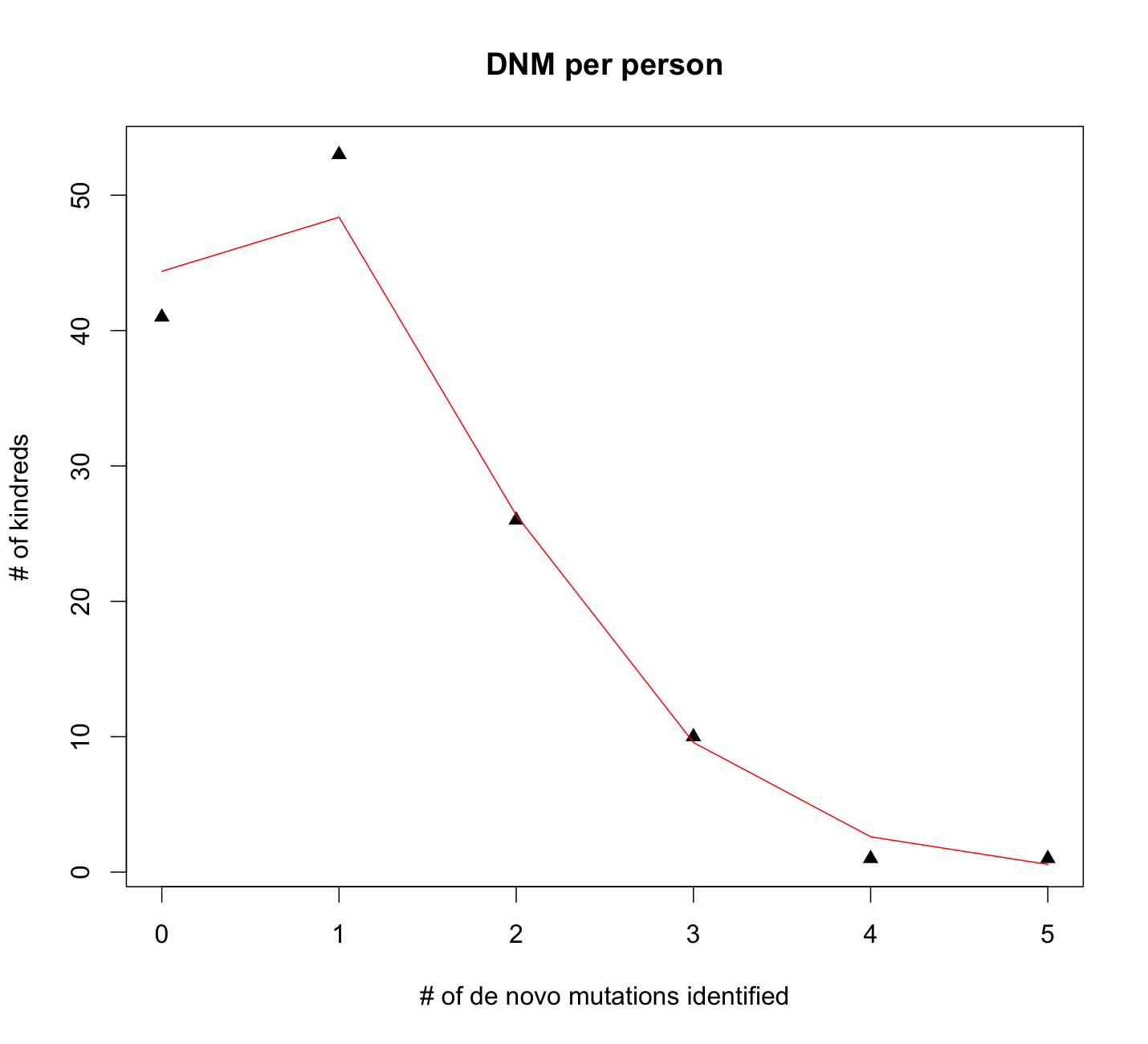
**Supplementary File 1E.**

**Clinical features and *BMP2* genotypes in craniosynostosis patients with rare *SMAD6*, *SMURF1*, *SPRY1*, or *SPRY4* mutations.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Kindred ID | Age (years) | Sex | Age at surgery | Suture(s) Involved | Mutation | *BMP2* Genotype | Other Phenotypes |
| SAGMET107-1 | 12 | M | 2.5yrs- late diagnosis | Sagittal and metopic | *SMAD6*: E287K, transmitted | C/C | -poor reading and spelling abilities, held back two years, frequent headaches, frequent complaints of bony thoracic pain |
| SAGMET101-1 | 1 | M | 4 months | Sagittal and metopic | *SMAD6*: Q78fs\*41 transmitted,  *de novo* in mother | T/C | -Meeting all milestones |
| SAGMET104-1  SAGMET104-2 | 5  1 | M  M | 6 months  5 months | Metopic  Sagittal | *SMAD6*: R345fs\*194, transmitted  *SMAD6*: R345fs\*194, transmitted | T/T  T/C | -No delays  -Meeting all milestones |
| SAG158-1 | 6 mos | M | 2.5 months | Sagittal | *SMAD6*: E374\*,  *de novo* | C/C | -Infant, hypospadias |
| SAGMET100-1  SAGMET100-2 | 2  30 | M  F | 4 months  No surgery | Sagittal and metopic  Sagittal and metopic | *SMAD6*: A353fs\*187, transmitted  *SMAD6*: A353fs\*187, transmitted | T/C  T/C | -Speech delay, early intervention for neurodevelopmental delay  -Lifelong headaches, some delays |
| MET127-1 | 5 | F | 5 months | Metopic | *SMAD6*: Q223\*, transmitted | T/C | -Speech delay, gross motor delay, fine motor impairment, language delays, persistent delays at age 5, asthma |
| MET148-1 | 6 mos | M | 3 months | Metopic | *SMAD6*: P323L, transmitted | T/C | -Infant |
| MET115-1 | 5 | M | 6 months | Metopic | *SMAD6*: I490T, transmitted | T/C | -Speech and motor delays, ankyloglossia, lip tie, inguinal hernia, retractile testis, asthma |
| MET111-1  MET111-2 | 15  15 | M  M | 1 year  1 year | Metopic  Metopic | *SMAD6*: R465C, unknown  *SMAD6*: R465C, unknown | T/C  T/C | - Global developmental delay, sensory disorder, marked speech delay, ADHD, inguinal hernia  - Global developmental delay, sensory disorder, marked speech delay, ADHD, inguinal hernia |
| MET154-1  MET154-2 | 6  6 | M  M | 6 months  opted against | Metopic  Metopic | *SMAD6*: E407\*, transmitted  *SMAD6*: E407\*, transmitted | T/C  T/C | -Early speech and motor delay  -Early speech and motor delay |
| Kindred ID | Age (years) | Sex | Age at surgery | Suture(s) Involved | Mutation |  | Phenotype |
| MET153-1 | 4 mos | F | 2.5 months | Metopic | *SMAD6*: G390C, *de novo* | T/T | -Infant |
| MET179-1 | 6 | M | 8 months | Metopic | *SMAD6*: S130fs\*146, transmitted | T/T | -Persistent motor delays |
| SAG220-1 | 7 | M | 17 months (late diagnosis) | Sagittal | *SMAD6*: R281fs\*13, transmitted | T/C | -No delays |
| SAG210-1 | 3 | F | 4 months | Sagittal | *SMAD6*: T306A, transmitted | T/C | -Speech delay until age 2.5 |
| SAGFAMILY1-1  SAGFAMILY1-2 | 9  5 | F  M | 4 months  4 months | Sagittal  Sagittal | *SPRY1*: Q6fs\*8,  *de novo* in mother  *SPRY1*: Q6fs\*8,  *de novo* in mother | C/C  C/C | -No other medical history  -Wolf-Parkinson-White syndrome evident at birth, recurrent episodes of V-Tach requiring cardioversion, ankyloglossia, lip tie |
| SAG150-1 | 9 mos | M | 7 months | Sagittal | *SPRY4*: E160\*,  *de novo* | C/C | -Infant |
| MET149-1 | 4 | F | 6 months | Metopic | *SMURF1*: R468W, *de novo* | T/C | -No other medical history |
|  |  |  |  |  |  |  |  |

*De novo* mutations are indicated. The primary inclusion criterion was LOF and damaging missense with allele frequency < 2 x 10-5 ; for completeness, the E287K variant with slightly higher allele frequency is included. For *BMP2* genotypes, alleles at rs1884302 are shown. The ‘C’ allele predisposes to midline craniosynostosis compared to the ‘T’ allele15.

**Supplementary File 1F.**

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***De novo* mutations identified per trio.** The number of *de novo* mutations identified per proband via exome sequencing of 132 case-parent trios was plotted (black triangles) alongside the expected Poisson distribution (red curve), demonstrating that the observed number of *de novo* mutations per proband closely matches expectation.