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**Supplemental Data**

# Mutations Causing Familial Biparental Hydatidiform Mole Implicate *C6orf221* as a Possible Regulator of Genomic Imprinting in the Human Oocyte

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Table S1. Results of SNP Genotyping

| **Chromosome** | **Physical coordinates of homozygous region (bp)** |
|---|---|
| 1 | 1-4006060 |
| 5 | 86097023-88953243 |
| 6* | 73877301-95872321 |
| 6 | 129876535-138417080 |
| 10 | 19362932-53502563 |
| 16 | 1-6167331 |

Six regions of concordant homozygosity in the two affected women from family L were identified by AutoSNPa analysis of Genome-Wide Human SNP Array 6.0 genotype data.

**C6orf221* is found on chromosome 6 at 74,072,400-74,073,896.

Table S2. Functional Variants Unique to Family L Identified Following Clonal Sequencing of Target Regions

| Chr/position | Ref | Alt | Gene | Variant | SIFT |
|---|---|---|---|---|---|
| 1:2523431 | G | T | *MMEL1* (NM_033467.3) | c.2103C>A;p.Asp701Glu | Tolerated |
| 6:74072455 | G | T | *C6orf221* (NM_001017361.2) | c.3G>T;p.Met1Ile | DAMAGING |
| 6:132874463 | T | A | *TAAR8* (NM_053278.1) | c.632T>A;p.Val211Asp | DAMAGING |
| 6:134583262 | T | C | *SGK1* (NM_001143676.1) | c.94A>G;p.Met32Val | Tolerated |
| 6:135360767 | A | T | *HBS1L* (NM_001145207.1) | c.374T>A;p.Val125Glu | Tolerated |
| 10:32740608 | A | G | *CCDC7* (NM_145023.4) | c.38A>G;p.Lys13Arg | Tolerated |
| 16:417691 | C | G | *MRPL28* (NM_006428.4) | c.755G>C;p.Arg252Thr | Tolerated |
| 16:2140581 | G | C | *PKD1* (NM_001009944.2) | c.12149C>G,p.Ser4050Cys | Tolerated |

Table S3. Primer Sequences for Generation of the Wild Type and Mutant Constructs using C6orf221 IMAGE Clone 40146866

| | Forward | Reverse |
|---|---|---|
| Wild type | ggttctcgagaCTGTGGTGTCCTTGTCTC | cctgagaagcttTAATCTAGTAACTGGGTCCC |
| Mutant | CCGGCCGCAGCATtGACGCTCCCAGGC | GCCTGGGAGCGTCaATGCTGCGGCCGG |

The original mutation ATG → ATT was recreated using the Quikchange kit (Stratagene) and these primers.

Table S4. Primers Used for Sequence Analysis *C6orf221*

| Exon | Forward | Reverse |
|---|---|---|
| 1 | AAATAAGGCCCAGGCAGAAC | GGAACGCAGCCAGAATATGT |
| 2 | ACCAGTAGCCAATGCCCTCT | GACTGGGAGGGCGAGACT |
| 3 | TCTGGGATTTCTGGCTCCTA | AGCTTCTGGGCGGAAATAC |

The forward and reverse primers used for PCR and Sanger sequencing of all the exons in *C6orf221* are indicated.

Table S5. *C6orf221* Mutations, Reproductive Outcomes and Ethnic Origin Of Women with FBHM. CHM, Complete Hydatidiform Mole; Misc, (8 Weeks Gestation); TOP, Termination of Pregnancy (6 Weeks Gestation); PTD, Persistent Trophoblastic Disease

| **Family** | **Affected patients** | **Mutations** | **Predicted protein changes** | **Reproductive outcome** | **Clinical outcome** | **Ethnicity** | **Ref** |
|---|---|---|---|---|---|---|---|
| L | L1 | c.3G>T/ c.3G>T | p.Met1Ile/p.Met1Ile | CHM × 8; Misc × 2 | PTD | Pakistani | 8 |
| | L2 | c.3G>T/ c.3G>T | p.Met1Ile/p.Met1Ile | CHM × 4 | - | Pakistani | 8 |
| T | T1 | c.322_325delGACT/ c.322_325delGACT | p.Asp108Ilefs*30/p.Asp108Ilefs *30 | CHM × 7; Misc; TOP | PTD | Tunisian | 24 |
| W | W1 | c.1A>G/ c.322_325delGACT | p.Met1Val/p.Asp108Ilefs*30 | CHM × 4 | PTD | Asian | 14 |

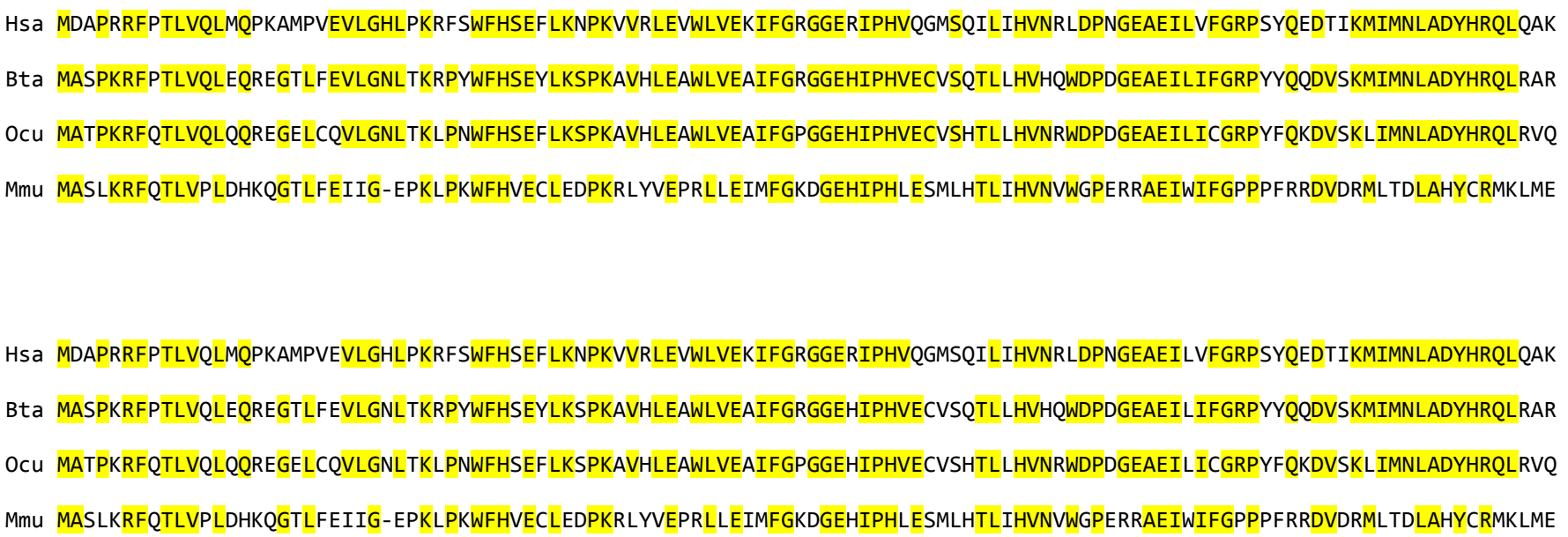


Figure S1. Protein Alignments of the First Two Exons of Human C6orf221 and the Bovine, Rabbit and Mouse Orthologs

Residues shared across three or four species are highlighted in yellow. Homo sapiens C6orf221, Bos taurus XM_002690018, Oryctolagus cuniculus XM_002714424, Mus musculus Filia.