## Supp. Methods

### Exome Sequencing and Analysis

All patient DNA samples were collected by local internal review board criteria, abiding by the Helsinki Treaty, and de-identified. Genomic DNA was isolated from peripheral blood samples using the Puregene Blood Kit (Qiagen; Valencia, CA). DNA quantification and quality assessment was performed using a NanoDrop spectrophotometer (Thermo Scientific; Wilmington, DE) and agarose gel electrophoresis. The Agilent SureSelect Version 4 (51Mb) exome enrichment kit was used prior to sequencing on an Illumina HiSeq 2000 instrument (Asan, et al., 2011; Bennett, 2004; Clark, et al., 2011; Parla, et al., 2011; Sulonen, et al., 2011). One hundred nucleotide long paired end reads were used to sequence each exome to an average coverage of approximately 50X before the removal of PCR duplicates.

The sequence analysis pipeline used for this study is based on the best practice variant detection guidelines developed by the Broad Institute for the Genome Analysis Tool Kit (GATK) suite of software utilities (McKenna, et al., 2010). The overall process can be described as three stages – alignment to the reference genome, identification of high-quality variations and genotypes, and annotation with biologically relevant information. Raw fastq files were quality controlled using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and aligned to the human genome reference sequence (build hg19) with BWA (Li and Durbin, 2009). The SAM/BAM standard format was used to store the alignment results (Li and Durbin, 2009). GATK (McKenna, et al., 2010), Samtools (Li and Durbin, 2009), and Picard tools (http://picard.sourceforge.net/) were used for single nucleotide variation (SNV) and small insertion and deletion (indel) detection and quality control filtering from the SAM/BAM files. This process included the removal of duplicate reads, local re-alignment of the reads, recalibration of base quality values, identification and genotyping of single nucleotide substitutions and small insertions/deletions (GATK Unified Genotyper), and variant quality score recalibration.

Prior to gene-content and functional annotation, we identified those variants that were found at greater than 1% in the 1000 Genomes Project (2010), dbSNP (Sherry, et al., 2001) (build 135),

and the Exome Variant Server (Wassink, et al., 2001). Furthermore, all variants were also compared to our own internal database of exome sequencing results from patients without the same phenotype. Following these initial variant filtration steps, annotation of remaining variants was performed using SnpEff and SnpSift (Cingolani, et al., 2012), the dbNSFP (Liu, et al., 2011), and the Human Gene Mutation Database Professional Version (HGMD) (Stenson, et al., 2003). HGMD was used to exclude a previously known disease causing mutation. The dbNSFP database was used to provide likely variant functional consequence with annotations from SIFT (Ng and Henikoff, 2003), Polyphen2 (Adzhubei, et al., 2010), LRT (Sul, et al., 2011), MutationTaster (Schwarz, et al., 2010), conserved domain sites from InterPro (Hunter, et al., 2012), GERP scores (Cooper, et al., 2005), and SiPhy scores (Garber, et al., 2009). Variation prioritization was accomplished by three methods 1) heuristic filtering based on suspected disease inheritance, 2) likely variant functional consequence (damaging variations such as nonsense and frameshift as well as nonconservative amino acid changes in highly conserved sites receiving higher priority), and 3) a probabilistic search algorithm, VAAST (Yandell, et al., 2011). Various quality control metrics were used throughout the analysis process. Fastq files were evaluated using FastQC, mapping was evaluated using both Samtools and Qualimap (to evaluate mapping performance and evaluate coverage of target, respectively) (Garcia-Alcalde, et al., 2012; Li and Durbin, 2009), and variant quality was assessed using parallel strategies of hard filtering (thresholds used were those recommended by the GATK Best Practices) and variant quality score recalibration. We assumed that any causative variant would be transmitted in a dominant manner and exhibit near complete penetrance. VarSifter (Teer, et al., 2012) was used to perform the inheritance based, variant segregation analysis via the custom query feature. VarSifter was also used to de-prioritize those variants that failed either hard filtering criteria or variant quality score recalibration. We retained any quality controlled variants that were found in 4 out of the 7 affected family members provided that in the other 3 samples there was insufficient information to make a call and not a discordant call. Those variants that fulfilled the hypothesized inheritance model were further prioritized according to in silico predictions of pathogenicity, nucleotide conservation, and presence within a conserved protein domain. These results were then intersected with those from the VAAST algorithm. VAAST was run assuming dominant inheritance, complete penetrance, and no locus heterogeneity. The results from VarSifter and VAAST were intersected and then sorted by the VAAST probability score and

variant functional consequence scores. A quality controlled, prioritized list of genes and variants that fit our hypothesized inheritance model is available in Supp. Table S1. The highest scoring variant/gene was the chr1:236201527 G>A variant in NID1 which produces a stop codon in place of a glutamine at amino acid position 388 (Q388*). This variant (NM_002508.2 (NID1_v001): c.1162C>T, p.(Gln388*)) was found in all 14 of the affected family members in pedigree 1 by Sanger sequencing. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1.

The NID1 nonsense mutation was not found in the proband from pedigree 2. Therefore, potential NID1 interacting proteins were ascertained from several different sources and the union was used to screen all rare variants from the exome sequencing analysis of this individual. Databases used included the commercial Ingenuity Pathway Analysis (IPA) Knowledge Base (www.ingenuity.com), STRING (Szklarczyk, et al., 2011), VisAnt (Hu, et al., 2009), and GeneMania (Warde-Farley, et al., 2010), and PUBMED search for interacting proteins. Lists obtained from these databases using default settings and the gene NID1 are available as Supp. Table S1. All rare coding variants (less than 1% frequency in databases mentioned above) within these genes were identified and assess for their potential pathogenicity in the proband from pedigree 2. The list of genes and variants matching these criteria in this individual are available in Supp. Table S2. Primers and conditions used for Sanger sequencing (all with Invitrogen platinum Taq) of *NID1* and *LAMC1* exons are listed in Supp. Table S3. We discovered a novel LAMC1 variant: NM_002293.3 (LAMC1_v001): c.2237C>T, p.(Thr746Met) (nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence with the initiation codon being codon 1). This variant was submitted to the LOVD LAMC1 at https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=LAMC1)

**Protein Structures**

We used the Yasara Structure (version 12.10.3) to generate a homology model of human LAMC1 EGF-like-6 domain (residues 722-770) using structures of other Laminin EGFlike

domains (pdb id: 1NPE, 3ZYG, 1KLO, 3TBD, 2Y38) as templates**.** Sequence alignment with the templates was first used to build a backbone model for aligned residues followed by loop modeling and side chain optimization using a combination of steepest descent and simulated annealing minimization. The top ranking of the 6 models generated was used as the homology model of LAMC1 EGF-like-6 domain. The above steps were automated using Yasara's hm_build macro (http://www.yasara.org). Another homology model was generated using the Phyre server (version 2.0) (http://www.sbg.bio.ic.ac.uk/~phyre), which showed agreement with the Yasara model (rmsd of 0.591 over 35 Cα atoms) for the domain. PyMOL (Schrodinger, LLC) was used to visualize the homology model and to generate figures.

**Supp. References**

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42.

**Supplementary Table 1: Potential NID1 Interacting Partners**

| Database Queried | IPA | | STRING | VisAnt | GeneMania | PUBMED PATHWAY | PUBMED CO-EXPRESSION |
|---|---|---|---|---|---|---|---|
| Genes | AHR | LACRT | CLIC1 | HSPG2 | NID1 | COL3A1 | FOXC1 |
| | BMI1 | LAMA1 | PLXDC1 | PRELP | HSPG2 | COL6A1 | PLXCD1 |
| | CHUK | LAMC1 | NID1 | MDK | LAMC1 | COL4A1 | ZIC |
| | COL18A1 | LAMC2 | HSPG2 | FBLN2 | COL4A1 | COL4A2 | ZIC2 |
| | COL1A1 | LAMC3 | COL4A1 | FBLN1 | COL4A2 | NID2 | ZIC3 |
| | COL4A1 | Laminin | PLAU | LAMA1 | PLXDC1 | SPARC | ZIC4 |
| | COL4A2 | Laminin1 | FBLN2 | SKIL | NID2 | KDR | |
| | ELN | LGALS3BP | FBLN1 | COL1A1 | FBLN2 | HSPG2 | |
| | ERBB2 | MDK | FN1 | LGALS3BP | ENPEP | | |
| | ERBB3 | MMP1 | | COL13A1 | COL3A1 | | |
| | ERBB4 | MMP12 | | FGB | LRRC17 | | |
| | FBLN1 | MMP14 | | FGA | COL18A1 | | |
| | FBLN2 | MMP2 | | LAMC2 | LACRT | | |
| | FGA | MMP3 | | LAMC1 | LAMC2 | | |
| | FGB | MMP7 | | COL4A1 | COL6A3 | | |
| | Fibrinogen | NFKBIA | | APP | SPARC | | |
| | FN1 | NID1 | | LACRT | CALD1 | | |
| | HRAS | NID2 | | PTPRF | SERPINH1 | | |
| | HSPG2 | PLXDC1 | | PLAU | LAMB1 | | |
| | IKBKB | PTPRF | | PLXDC1 | FBLN1 | | |
| | IKBKG | RHO | | ITGAV | PDGFRB | | |
| | IL13 | SERPING1 | | NID1 | | | |
| | ITGA3 | SKIL | | ITGB3 | | | |
| | ITGB1 | TNF | | | | | |

**Supplementary Table 2: Rare Coding Variants within Genes that Encode Proteins Predicted to be NID1 Interacting Partners**

| Chr | LeftFlank | RightFlank | Gene_name | type | muttype | ref_allele | var_allele |
|---|---|---|---|---|---|---|---|
| chr3 | 13613080 | 13613082 | FBLN2 | DOWNSTREAM;NON_SYNONYMOUS_CODING | SNP | C | T |
| chr1 | 22167603 | 22167605 | HSPG2 | UPSTREAM;NON_SYNONYMOUS_CODING | SNP | G | C |
| chr18 | 6956510 | 6956512 | LAMA1 | UPSTREAM;NON_SYNONYMOUS_CODING | SNP | C | T |
| chr9 | 133962979 | 133962981 | LAMC3 | NON_SYNONYMOUS_CODING | SNP | C | T |
| chr1 | 183091221 | 183091223 | LAMC1 | UPSTREAM;NON_SYNONYMOUS_CODING | SNP | C | T |
| chr18 | 7033037 | 7033039 | LAMA1 | NON_SYNONYMOUS_CODING | SNP | G | A |
| chr22 | 45944567 | 45944569 | FBLN1 | NON_SYNONYMOUS_CODING | SNP | A | G |

| **Supplementary Table 3: *NID1* and *LAMC1* PCR primers and annealing Temperatures** | | | |
|---|---|---|---|
| **NID1** | **direction** | **primer sequence** | **annealing Temp** |
| Exon 1 | Forward | GTTACATCCCCGCCTTCCT | 60ºC |
| | Reverse | CCTGCTACGCCCAAGTCC | |
| Exon 2 | Forward | AGCATGGGTACAGCTGACAGT | 60ºC |
| | Reverse | CACAAGGCGTGAGACCAAAG | |
| Exon 3 | Forward | TGAGAATATTGCCATCGCTCT | 60ºC |
| | Reverse | TGCAAAACTTAAATCCATAGGAA | |
| Exon 4 | Forward | TTGCAGACCAACACAGATCC | 60ºC |
| | Reverse | AACTGACATCTCCCCAGCAG | |
| Exon 5 | Forward | AGTGCTTGAGGGTGCCTAGA | 60ºC |
| | Reverse | TGTGGGTAAAGCAACATGGA | |
| Exon 6 | Forward | GACCCCAGTGCTGTTTTGTT | 60ºC |
| | Reverse | GAGTTCTCCTTCCATCCATCC | |
| Exon 7 | Forward | ACCAACCCCACTCCACACT | 58ºC |
| | Reverse | CTCCAGATCCCAGAGACAGC | |
| Exon 8 | Forward | TTATGGGCAGGGAATACCAC | 60ºC |
| | Reverse | TTTTTCCTGCCAGAAGATACTTT | |
| Exon 9 | Forward | AGTTGTGGTTGACTTTCTGTGG | 60ºC |
| | Reverse | GGTTTACAGAACGTGGATGC | |
| Exon 10 | Forward | GCAAGACTCCATCTCGGAAA | 60ºC |
| | Reverse | CAGCACCTTCCAACTTGACC | |
| Exon 11 | Forward | TCCAGGCATGGTCTCTCTTT | 60ºC |
| | Reverse | TGAGTTGGTGATTGGCACAT | |
| Exon 12 | Forward | TTTGAGTAAGTGGCTTAAATTATTGG | 60ºC |
| | Reverse | ATGGACAGGGCAGGCAAT | |
| Exon 13 | Forward | TGATGAAACAAAACCCAGTGA | 60ºC |
| | Reverse | GGTCCAGCAGAAGAGCAAAG | |
| Exon 14 | Forward | ACCTGATGTGTGGGTGTGTG | 60ºC |
| | Reverse | CCTGGGGTTCTGGTCTGATA | |
| Exon 15 | Forward | CAAAAGATATCCAAATGCCTGT | 60ºC |
| | Reverse | TTTGGCTTCATTTGCTGTTC | |
| Exon 16 | Forward | GGATTGGACACCATGTGAAA | 60ºC |
| | Reverse | CAGGAGACCAATGCTGGAGT | |
| Exon 17 | Forward | TGTTTTCATGTCTGCCAATCA | 60ºC |
| | Reverse | CAGGTGGCTGGTTAGATTTG | |
| Exon 18 | Forward | CCACTCTTGCCCTCATTACTC | 58ºC |
| | Reverse | AGAGGCCAGATGGGAAGG | |
| Exon 19 | Forward | TGGCCTCCAGTTTATCCTCA | 60ºC |
| | Reverse | CATACGGGTAGCAGCCAGAG | |
| Exon 20 | Forward | CACACAAGAAGGAGCCCACT | 60ºC |
| | Reverse | CAGCCAGAGGACACTTTTCA | |

| LamC1 | direction | primer sequence | annealing Temp |
|---|---|---|---|
| Exon1A | Forward | CAGGCTCAAGCAGCGAAG | 56˚C |
| Exon1A | Reverse | CGTCGCACAGGTGACAGG | |
| Exon1B | Forward | ATGCCCGAGTTCGTCAAC | 58˚C |
| Exon1B | Reverse | GCTGGCGAGCAGTAGCAG | |
| Exon2 | Forward | CCAAACTAAGCTGGGAATAGAGG | 58˚C |
| Exon2 | Reverse | GCTCTCTGTCTGTGTTGCTCA | |
| Exon3 | Forward | GTGCTAAAGAGGGCGTAAGTT | 58˚C |
| Exon3 | Reverse | TGCAAAAGCTCCAAACACTTT | |
| Exon4 | Forward | GGACTTTGCAGCTGTTCCAT | 58˚C |
| Exon4 | Reverse | GGCCTTTCAGAGCTTTCCTT | |
| Exon5 | Forward | TCTGTCTCAGAGATGTGGGAAAT | 58˚C |
| Exon5 | Reverse | GTCCAAATGCCTTGGAAACA | |
| Exon6 | Forward | GGCCGAATTTTTGTTTGACA | 58˚C |
| Exon6 | Reverse | TTACCCTGCTGCCAATAAGC | |
| Exon7 | Forward | TGTAAAACAAAGGGCTGACTTG | 60˚C |
| Exon7 | Reverse | TTGAAATATGGTAACAGAAAACACA | |
| Exon8 | Forward | TGTCGAAGGCTTCAATTGTG | 58˚C |
| Exon8 | Reverse | CAAAAAGCCCTCAAAGAGTCA | |
| Exon9 | Forward | TTTTTATGATACATAGAGGACCTGAA | 58˚C |
| Exon9 | Reverse | TGGGCAAGAATGTGAGACTG | |
| Exon10 | Forward | CAGTCTGACCTGCTGTGTGC | 58˚C |
| Exon10 | Reverse | CACCACAATAACCCAGAAGTTTT | |
| Exon11 | Forward | TCCAGAATTGTCCTTACAGAGG | 58˚C |
| Exon11 | Reverse | GGAAAGAGAAACTTTTGCATTATCA | |
| Exon12 | Forward | TCTGGAATTTGGGGTCTAGTT | 56˚C |
| Exon12 | Reverse | AAGACAATGAGCACTTTCTACACAA | |
| Exon13 | Forward | ATATACTTGTACATTTGCCTGTTTTC | 58˚C |
| Exon13 | Reverse | CCCCTTTCCCCTAGGTCTTT | |
| Exon14 | Forward | CCAGTGGTAATCTAGCACCAAA | 58˚C |
| Exon14 | Reverse | AAGAGACACACAAGAATTCTCACAA | |
| Exon15 | Forward | CAGCTACCAACCTGGCAAC | 58˚C |
| Exon15 | Reverse | GCAAAGATTACTTTCACCAAAGG | |
| Exon16 | Forward | CCATTCAGTTTTCTGATACAAGTTACA | 58˚C |
| Exon16 | Reverse | GGCTAGACTGAGGCTGTCAAA | |
| Exon17 | Forward | TCTCTGCTAAACAGACTTAGCCATT | 56˚C |
| Exon17 | Reverse | CAGTAAGTTGCCACTCAACGTC | |
| Exon18 | Forward | TGTGTGTCCTACCTTTCCCTTT | 58˚C |
| Exon18 | Reverse | TGGTCCATCTCACAGAAGTCA | |
| Exon19 | Forward | TGAGATGATATGATCCTCTTCAGAT | 58˚C |
| Exon19 | Reverse | CATCCCCACCTCAGACGTT | |
| Exon20 | Forward | AATGTAAATAATTCAGTCCTTTGAGAA | 56˚C |
| Exon20 | Reverse | ACCCCATAACAGAAGCCACA | |
| Exon21 | Forward | CCTGAATTTTACTTATGTCCCTATCA | 58˚C |
| Exon21 | Reverse | AGGGGTGTAAACCACTTTCAA | |
| Exon22 | Forward | TGCTAAAAGCAGCTAAGATTGTCA | 58˚C |
| Exon22 | Reverse | ATAGACTTGGGGGAGAGACG | |
| Exon23 | Forward | TGCAGTTCTTTTAAAAATATCATTGAG | 58˚C |
| Exon23 | Reverse | CTCAAGAAATGAAGCGACCTG | |
| Exon24 | Forward | GAGCCTTGTCTGTCCAGGAG | 58˚C |
| Exon24 | Reverse | CACTCTAGTGGCGGAAGCAT | |
| Exon25 | Forward | ACCCTGACTTCCATTGTTCA | 56˚C |
| Exon25 | Reverse | GCTCTGAGGGTCTGGAAAGA | |
| Exon26 | Forward | AGCGGGAGCATACTTGAGAA | 56˚C |
| Exon26 | Reverse | TCAACAAGTGGGGGATCTTT | |
| Exon27 | Forward | TTAGATTTGTTGGGTCATTGG | 58˚C |
| Exon27 | Reverse | CAAAAGATTCAGAGACTGACAAAAA | |
| Exon28 | Forward | CAGGTACTTGTGAAGGGATCATT | 58˚C |
| Exon28 | Reverse | ACTCTGTGGCCTCACAACTG | |