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Dsp^{rul}: A spontaneous mouse mutation in desmoplakin as a model of Carvajal-Huerta syndrome

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ABSTRACT

Studies of spontaneous mutations in mice have provided valuable disease models and important insights into the mechanisms of human disease. Ruffled (*rul*) is a new autosomal recessive mutation causing abnormal hair coat in mice. The *rul* allele arose spontaneously in the RB156Bnr/EiJ inbred mouse strain. In addition to an abnormal coat texture, we found diffuse epidermal blistering, abnormal electrocardiograms (ECGs), and ventricular fibrosis in mutant animals. Using high-throughput sequencing (HTS) we found a frameshift mutation at 38,288,978 bp of chromosome 13 in the desmoplakin gene (*Dsp*). The predicted mutant protein is truncated at the c-terminus and missing the majority of the plakin repeat domain. The phenotypes found in *Dsp*^{rul} mice closely model a rare human disorder, Carvajal-Huerta syndrome. Carvajal-Huerta syndrome (CHS) is a rare cardiocutaneous disorder that presents in humans with wooly hair, palmoplantar keratoderma and ventricular cardiomyopathy. CHS results from an autosomal recessive mutation on the 3′ end of desmoplakin (*DSP*) truncating the full length protein. The *Dsp*^{rul} mouse provides a new model to investigate the pathogenesis of CHS, as well as the underlying basic biology of the adhesion molecules coded by the desmosomal genes.

1. Introduction

Desmosomes are one of the major cell–cell adhesion complexes, along with adherens, tight and gap junctions (Brooke et al., 2012; Gallicano et al., 1998). The desmosomes consist of three main entities: 1) intercellular desmosomal cadherins (desmocollin and desmoglein), 2) intracellular linker proteins called plakoglobin and plakophillin, and 3) intracellular cytoskeletal linker called desmoplakin (Brooke et al., 2012; Gallicano et al., 1998). The strong cell–cell adhesive bond afforded by the desmosomes is important in maintaining the integrity of epithelia and the resistance to mechanical forces experienced by cardiomyocytes (Vasioukhin et al., 2001). Desmoplakin functions to link the desmosomal complex to intermediate filaments of the cytoskeleton, such as the keratins (Brooke et al., 2012; Gallicano et al., 1998). Previous work has shown that DSP is expressed in all layers of the epidermis (Bierkamp et al., 1999), as well as in the hair follicle outer root sheath, companion layer, and in basal areas of Henle's and Huxley's layers (Alibardi and Bernd, 2013; Kurzan et al., 1989). It is also a critical part of the intercalated discs binding cardiomyocytes together in the heart (Brooke et al., 2012; Gallicano et al., 1998). Human genetic lesions affecting the function of *DSP* result in a number of disease including dilated cardiomyopathy with wooly hair, keratoderma and tooth agenesis (DCWHKTA; OMIM #615821) (Norgett et al., 2006), familial arrhythmogenic right ventricular dysplasia 8 (ARVD8; OMIM #607450) (Rampazzo et al., 2002), lethal acantholytic epidermolysis bullosa (OMIM #609638) (Jonkman et al., 2005) and Carvajal-Huerta syndrome (OMIM #605676) (Carvajal-Huerta, 1998).

We report here a new spontaneous recessive mouse mutation that arose in 1989 in the RB156Bnr/EiJ recombinant inbred strain at The Jackson Laboratory. The phenotype manifests as an abnormal, ruffled coat texture that appears at 10 days of age and is maintained through the life of the animal, hence the allele name, *ruffled* (*rul*). In mutant animals, we observed sporadic epidermal blistering, intermittent pelage hair swelling, widespread vibrissa follicular dystrophy, ventricular fibrosis and electrocardiographic abnormalities. Using high-throughput sequencing, we found that *rul* is a mutation in mouse desmoplakin (*Dsp*) gene. While targeted alleles of *Dsp* have been previously reported, *rul* is the first known spontaneously arising mutation in *Dsp*. The mutant gene and the phenotype of RB156Bnr/Ei-*rul*/GrsrJ mice support this

Abbreviations: CHS, Carvajal-Huerta syndrome; *rul*, ruffled, *Dsp*/DSP, desmoplakin gene/protein; tm, targeted mutation; HTS, high throughput sequencing; kD, kilodalton.

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strain as a new model of human syndromes that involve integumentary and cardiac defects like Carvajal-Huerta syndrome.

2. Materials and methods

2.1. Mice

Phenotypically deviant mice with ruffled coat texture were identified in the RB156Bnr/EiJ inbred strain at The Jackson Laboratory (Bar Harbor, ME). Affected animals were crossed to the C57BL/6EiJ inbred strain to test heritability of the allele. No affected animals were observed in the F1 generation, but affected F2 animals were obtained from subsequent intercrosses between F1 progeny, demonstrating a recessive mode of inheritance for the *rul* allele. Both male and female *rul*/*rul* homozygotes were fertile, and the mutant strain, RB156BNR/Ei/J-*rul*/J has been maintained since 1989 by crossing homozygotes with phenotypically unaffected heterozygotes. The treatment and use of all mice in this study were compliant with protocols approved by The Jackson Laboratory Animal Care and Use Committee at the Mouse Mutant Resource (MMR) of The Jackson Laboratory (Bar Harbor, ME, USA).

2.2. Genetic linkage analysis

The *rul* mutation was genetically mapped in an intercross with CAST/EiJ. CAST/EiJ inbred mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). A *rul* homozygous mouse was mated with a wild type CAST/EiJ mouse. The phenotypically normal F1 progeny from this mating was then intercrossed and affected (*rul*/*rul*) mice were genotyped using strain-specific microsatellite markers to distinguish CAST/EiJ alleles from C57BL/6EiJ alleles across all chromosomes.

2.3. High-throughput sequencing

Whole exome sequencing was used to identify candidate mutations in the mapped region, as described previously (Fairfield et al., 2011). Briefly, genomic DNA was prepared using a Qiagen DNeasy blood and tissue kit (Qiagen, Santa Clarita, CA USA) or by phenol/chloroform extraction. The DNA was enriched for coding sequence by hybridization-based capture using a SeqCap EZ Mouse Exome SR (Roche NimbleGen). Post-hybridization amplification was completed via an Illumina LMPCR protocol as previously described (Fairfield et al., 2011). The resulting enriched libraries were used in a cluster formation on an Illumina cBot (Illumina, San Diego, CA). Paired-end sequencing was done using the Illumina HiSeq. The sequencing data were analyzed using tools and workflows provided by GenomeQuest including mapping (HS3), SNP calling, and annotation of variants (GenomeQuest, Boston, MA). Analysis focused on novel variants, which were not positioned in repetitive sequence, and had expected allele ratios (>0.95 for homozygous variants and >0.2 for heterozygous variants), as well as sufficient locus coverage (at least 5× for homozygous variants and 10× for heterozygous variants). Protein coding or splice variants within the mapped interval that were unique when compared to ~100 unrelated exome data sets and inbred strain, variant data generated by the Sanger Mouse Genomes Project (Keane et al., 2011; Mouse Genomes Project) were flagged as candidate mutations. High priority was given to protein coding or splice variants within mapped regions, as well as unique variants that were not found in other exome data sets or in data generated by the Sanger Mouse Genomes Project (Keane et al., 2011; Mouse Genomes Project).

2.4. Mutation validation

Candidate mutations were validated by PCR amplification and capillary sequencing of homozygous *rul*/*rul* and heterozygous *rul*/+ genomic DNA samples. Sequencing data were analyzed using Sequencher 4.9 (Gene Codes Corp., Ann Arbor, MI, USA). Primers rul_Dsp1L (AGGAAG ACCGGCAGTCAGTA), rul_Dsp1R (GGGAGAGCTTCTGACCAGTG) and rul_Dsp2L (CGACACGACTCCGTGAGTAA), rul_Dsp2L (CTTCTTCGGTGC TGATCCTC) were designed using Primer3 software (Rozen and Skaletsky, 2000). PCR reactions were performed using the following cycling conditions: preheating at 95 °C for 3 min; 40 cycles of amplification at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. PCR products were purified by The Jackson Laboratory's Genome Sciences group using Agencourt's AMPure XP magnetic beads (Agencourt, Bioscience Corp., Beverly, MA). Sanger sequencing reactions were set up using 5 μL of purified PCR product and 1 μL of primer at 5 pmol/μL. Cycle sequencing of DNA samples was performed using an ABI BigDye Terminator ready reaction kit v3.1 and ran on an ABI 3730xl (Applied Biosystems, Carlsbad, CA). Sequence reads were analyzed and aligned using Sequencher software (Gene Codes Corp., Ann Arbor, MI).

2.5. Electrocardiogram (ECG)

Cardiac electrical activity was recorded from conscious mice gently restrained in a Plexiglas cylinder using metal pads to collect the signal. The animals were restrained in a Murine ECG Restrainer (Emka Technologies, Paris, France), and the signal acquisition used a PowerLab System (AD Instruments, Golden CO). The QRS Phenotyping system consists of an ECG platform with a conductive metal for foot contact and a restrainer tube, with a small breathing hole and an adjustable tail gate. There are three different cylinder sizes to accommodate different size animals. The tailgate sizer is used to limit the lateral space within the restrainer (to prevent the mouse from turning around) and is adjusted so that the animal's front feet and left hind foot are positioned on the appropriate pad. The tail is able to exit the restrainer through a slit in the tail gate. Mice were housed in the testing room overnight for acclimation before testing. After the mouse was gently placed in chamber, the ECG signal was recorded for 5 min or until a steady baseline signal was recorded for a 20 to 60 second interval. Some mice take longer to acclimate and settle down, in which case they were left in the chamber for a longer period. Mice were returned to their home cage and then euthanized for histopathological analysis of the heart.

2.6. Statistics

All statistics were carried out on ECG data using the statistics program JMP v11 (SAS International, Cary, NC). A Shapiro–Wilk test was used to determine normality of the data with the assumption that normally distributed data will give a p-value > 0.05. RR interval ($p = 0.4812$), QRS ($p = 0.0803$), heart rate ($p = 0.2607$), QT interval ($p = 0.1739$), QTc ($p = 0.6663$), P amplitude ($p = 0.1060$), Q amplitude ($p = 0.3790$), S amplitude ($p = 0.0615$), and T amplitude ($p = 0.5716$) data were found to be normally distributed, while PR interval ($p = 0.0163$), P duration ($p = 0.0085$), and R amplitude ($p = 0.0012$) were not normally distributed. RR interval, QRS interval, heart rate, QT interval, QTc, P amplitude, Q amplitude, S amplitude, and T amplitude data were analyzed using a Student's t-test, while PR interval, P duration, and R amplitude data were analyzed by Mann–Whitney tests. p-Values of less than 0.05 were considered significant for all statistical tests.

2.7. Histology

To define histological lesions, 12 week old $Dsp^{rul/+}$ and $Dsp^{rul/rul}$ animals were heavily sedated by interperitoneal injection of 2% Tribromoethanol (600–800 mg/kg) until no toe pinch reaction was observed. Animals were then perfused with Bouin's solution by intracardiac perfusion. After perfusion animals were immersed in a Bouin's solution until tissue harvest. Tissues, including brain, spinal cord, thyroid, thymus, heart, lung, liver, diaphragm, kidney, adrenal glands, spleen, intestines, urinary bladder, skin, testis, uterus, ovary, hind legs, tail, and foot were washed overnight, trimmed, processed and embedded routinely in paraffin, sectioned, and stained with hematoxylin and eosin