Cloning of the Gene for Human Pemphigus Vulgaris Antigen (Desmoglein 3), a Desmosomal Cadherin

CHARACTERIZATION OF THE PROMOTER REGION AND IDENTIFICATION OF A KERATINOCYTE-SPECIFIC cis-ELEMENT*

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Pemphigus vulgaris antigen is a cadherin-like desmosomal cell adhesion molecule expressed primarily in suprabasal keratinocytes within the epidermis. Previously characterized structural features have defined this molecule as a desmoglein, DSG3. In this study, we have cloned the human DSG3 gene and examined the transcriptional regulation of its expression. The total gene consisted of 15 exons and was estimated to span 23 kilobases. Comparison of exon-intron organization of DSG3 with bovine DSG1 and several classical cadherin genes revealed striking conservation of the structure. Up to 2.8 kilobases of the upstream genomic sequences were sequenced and found to contain several putative cis-regulatory elements. The promoter region was GC-rich and TATA-less, similar to previously characterized mammalian cadherin promoters. The putative promoter region was subcloned into a vector containing chloramphenicol acetyl transferase reporter gene. Transient transfections with a series of deletion clones indicated that the DSG3 promoter demonstrated keratinocyte-specific expression, as compared with dermal fibroblasts examined in parallel, and fine mapping identified a 30-base pair segment at -200 to -170 capable of conferring epidermal specific expression. The results provide evidence for the transcriptional regulation of the pemphigus vulgaris antigen gene, potentially critical for development of the epidermis and physiologic terminal differentiation of keratinocytes.

Pemphigus vulgaris is an acquired blistering skin disease characterized by circulating IgG autoantibodies in the serum of affected individuals (1). These circulating IgG antibodies have been shown to be pathogenetic by intracutaneous injection of the IgG fraction into the skin of neonatal mice with resultant intraepidermal blistering (2). Similarly, dysadhesion of the epidermal keratinocytes has been demonstrated in a skin organ culture system by incubation with pemphigus vulgaris sera (3, 4). Therefore, the patients' sera were used to characterize the antigen responsible for epidermal cell adhesion. Early immunofluorescence studies demonstrated that these antibodies recognize epitopes in the intercellular space within stratifying squamous epithelia, such as the human epidermis (5). Immunoblot analyses identified a 130-kDa glycoprotein, which was designated as pemphigus vulgaris antigen (PVA) (6, 7). Immunoelectron microscopic studies localized the PVA epitopes to the extracellular portion of keratinocyte desmosomes within the epidermis (7). Understanding of the molecular structure of PVA was furthered by isolation of cDNAs corresponding to human PVA sequences (8). A partial PVA cDNA was isolated from a λgt11 expression library by immunoscreening using serum from a patient with pemphigus vulgaris. This cDNA was used to screen human epidermal keratinocyte cDNA libraries, resulting in the isolation of two additional overlapping cDNAs, which delineated an open reading frame of 2,997 bp of the mRNA (8). Examination of the deduced amino acid sequences, without post-translational modification, suggested that PVA mRNA encodes a 115-kDa polypeptide with homology to the desmoglein family of desmosomal transmembrane adhesion proteins and was subsequently designated as desmoglein 3 (DSG3) (8, 9).

The desmogleins are members of the cadherin supergene family, sharing several features with classical cadherins (10). Thus, PVA is a cadherin-like desmosomal cell adhesion molecule that demonstrates tissue-specific expression, as determined at the protein and mRNA levels. However, the mechanisms responsible for the tissue-specific expression of this gene or the cis-elements regulating its expression have not been delineated. In this study, we have cloned human PVA genomic sequences, delineated the entire exon-intron organization, and characterized the promoter region. We have also identified a 30-bp cis-element at -200 to -170 that contributes to the keratinocyte-specific expression of PVA.

MATERIALS AND METHODS

Isolation and Characterization of PVA Genomic Clones—To isolate human PVA genomic clones, a 468-bp cDNA was generated by RT-PCR using a commercial kit (Life Technologies, Inc.). RNA was isolated from...
cultured human keratinocytes (11), and a primer D3 (see Fig. 5) corresponding to positions 529–509 was used for reverse transcription using avian myeloblastosis virus reverse transcriptase. Two primers, PUI at -73 to -53 (5'-TAGAGCCCAGCATGTCG-3') and D4 at 395-375 (5'- CATTAACTGCAGACGGCTGC-3') were used to PCR amplify the cDNA. The corresponding 468-bp product was subcloned into the PCR vector, pGEM 7Z (Promega, San Diego, CA). Calcium-dependent transfection of the inserts was radioactively labeled by nick translation and used to screen vector, PCR II (Invitrogen, San Diego, CA), and sequenced (12). This primer extension analyses were performed (Promega PE kit) to determine the promoter. Subsequent characterization of these clones by restriction enzyme digests revealed that they represented four unique clones. These clones ranged in size from 15.7 to 18.3 kb and were determined by agarose gel electrophoresis of the inserts. The relative alignment of the genomic clones was determined by restriction enzyme digests with BamHI, HindIII, XbaI, EcoRI, Sad, and Sall, under conditions recommended by the manufacturer (IBI, New Haven, CT). The restriction enzyme digests were analyzed on 0.8% agarose gel electrophoresis and subsequently by Southern hybridization using PVA cDNA-specific oligonucleotide primers (12). A 3.4-kb XbaI fragment containing the promoter (see “Results”) was subcloned into the Bluescript (Stratagene) vector and sequenced in both directions using standard techniques (13) (U.S. Biochemical Corp.). The nucleotide sequence data were analyzed for eukaryotic promoter consensus sequences using a computer program, SIGNAL (pdm). DNA Sequence Analysis of the Exon-Intron Organization—Direct cycle sequencing (Amplicita, Perkin-Elmer, Norwalk, CT) of genomic clones with cDNA-specific primers permitted identification of the exon-intron borders. These analyses identified 11 exons, which corresponded to ~50% of the 5'-half of the coding region of PVA cDNA (8). To identify the remaining exons, total genomic DNA, isolated from peripheral blood leukocytes, was used as template for PCR amplification using primers designed on the basis of cDNA sequences. The PCR products were subjected to cycle sequencing using the same primers in an automated nucleotide sequencer (ABI). Northern Analysis—Total RNA for Northern blot analysis was isolated from cultured normal human keratinocytes (NHKs) (11) and hybridized to a 468-bp PVA cDNA probe under standard conditions (12). For comparison, parallel filters were hybridized with BPAc1 and BPAg2 cDNAs (14).

Determination of Transcription Initiation Sites—Two complementary approaches were taken to determine the 5'-end of the PVA mRNA. First, primer extension analyses were performed (Promega PE kit) using an antisense 20-bp oligomer corresponding to nucleotide positions -3 to -8 (5'-TCGCAATCCTCCATGAACCA-3'), which was extended from the 5'-end of the mRNA using avian myeloblastosis virus reverse transcriptase. The radioactive primer extension products were fractionated on 8.0% polyacrylamide gels, and autoradiograms were developed by exposure of the gel to x-ray film. The sizes of the bands were estimated by comparison with the radioactive molecular weight markers 4x174 DNA HindIII included in the kit.

The second approach to determine the transcription initiation site was to develop a first-strand cDNA from keratinocyte RNA using an antisense 21-bp oligomer, D3 (see Fig. 5), in the position extending from 529 to 509. The newly synthesized cDNA was then used as a template for PCR amplification with a series of primer pairs that all consisted of a common downstream primer, D1, in positions 89–70, and an upstream primer that was placed in different locations in the 5'-flanking region. To delineate the transcription initiation site, parallel amplification of genomic sequences was performed utilizing the same set of the upstream primers as used for amplification of the cDNA. However, since the first intron was found to be relatively large (~6.5 kb), the newly downstream primer was placed at the 5'-end of the intron 1 (the sequence of this primer, D2, was 5'-TCGCAATCCTCCATGAACCA-3'). The conditions for PCR amplification were as follows: 94°C for 4 min followed by 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 38 cycles utilizing Taq polymerase (Perkin-Elmer) and a thermal cycler (Omnigen, Marsh Biomedical, Rochester, NY). The amplification products from cDNA and genomic DNA templates were examined by 1.5% agarose gel electrophoresis.

Development of PVA Promoter/ CAT Constructs—Two of the genomic phase clones (see Fig. 1, numbers 1 and 2) were shown to contain the 5'-end of the coding region, as well as flanking DNA sequences (see “Results”). To examine the functionality of the putative promoter region within the 5'-end of the PVA gene, a series of promoter/CAT reporter gene constructs were developed. Specifically, PCR amplification products were synthesized using clones 1 or 2 as templates and primers that extended from position –1 upstream to positions –2,300, –1,200, –600, –500, –400, –300, –260, –230, –200, and –160, respectively. The upstream primers were designed to contain a restriction enzyme site for XbaI, and the downstream primer was designed with a restriction enzyme site for HindIII, which allowed direct insertion of the PCR products into the promoterless CAT construct, AceB (U.S. Biochemical Corp.). The restriction enzyme sites for HindIII and BamHI were cloned in front of a TK-CAT construct (pBlCAT2). The kinase-specific restriction site was confined to –200 to –170, and subsequently, four additional constructs containing 2-bp substitutions in the putative OX binding site in this region were developed.

Transient Cell Transfections and CAT Assays—The newly synthesized PVA promoter/CAT constructs were used for transient transfections of primary cultures of NHKs or normal human fibroblasts (NHFs) as described elsewhere (14).

Following transfection, the cells were incubated for 36 h at 37°C, and cellular proteins were isolated and used for assay of CAT activity (14). CAT activity was determined as the percentage of the acetylated form of chloramphenicol relative to total radioactive chloramphenicol in the sample. The CAT activities were corrected for the amount of protein in each cell extract.

RESULTS

Cloning Strategy of Human PVA Genomic DNA—PVA genomic clones were isolated using a 468-bp cDNA probe corresponding to the 5'-end of mRNA. The probe was used to screen a human lung fibroblast XFIXII genomic DNA library. Initial hybridization identified four unique clones (clones 1–4), which contained inserts of 18.5, 15.7, 16.1, and 16.0 kb in size, respectively (Fig. 1). These clones were purified and subjected to further analysis by restriction enzyme digestion and nucleotide sequencing. Enzyme digestions with a variety of restriction endonucleases (see “Materials and Methods”) allowed alignment of these four clones (Fig. 1). The results indicated that clones 1 and 2 were overlapping with each other, while clones 3 and 4 contained overlapping sequences. On the other hand, there was no overlap between clones 1 and 2 with either clone 3 or 4, as determined by restriction enzyme site mapping (Fig. 1). The total genomic DNA distance contained within these clones is approximately 40 kb.

Exon-Intron Organization of the Gene—The 5'-phase clone 1 was sequenced with primers corresponding to the 5'-end of the open reading frame as well as to the 5'-untranslated region of the cDNA sequence, previously published (8). An exon-intron border was observed 46 bp downstream from the translation initiation site, where the genomic clone sequence diverged from the cDNA sequence. This border was subsequently shown to be the exon 1/intron 1 border (see below). Similar sequencing of clones 3 and 4 with primers that corresponded to the cDNA sequences downstream from exon 1 identified 10 additional exons (numbers 2–11) (Fig. 1). Comparison of the sequences encoded by the 11 exons revealed that they corresponded to approximately 50% of the coding region of PVA cDNA. To identify the remaining exons, total human genomic DNA was used as template for PCR amplification utilizing primers synthesized on the basis of coding sequences in the 3'-half of the gene. Sequencing of the PCR products identified four additional exons, and therefore DSG3 is composed of a total of 15 exons (Fig. 1). The sites of the exons, as determined by nucleotide sequencing and by comparison with the open reading frame in the cDNA (8), varied from 33 to 864 bp (Table I). All exons were flanked by consensus 5'-donor and 3'-acceptor splice sites (Table I).

The sizes of the introns were estimated from the size of PCR products generated by amplification of genomic DNA by prim-
ers placed on adjacent exons. Using this methodology, the sizes of introns 2–14 were estimated to be −0.3 to −2.8 kb (Table I). No PCR product was obtained when attempts were made to amplify intron 1 sequences. On the basis of sequence information on clones 2 and 3, the size of intron 1 was determined to be >6.5 kb (see “Discussion”). Based on combined sizes of the introns and exons, the entire human PVA gene is estimated to be >23 kb.

Characterization of the 5'-End and the Flanking Region of the Gene—To identify putative cis-regulatory elements within the 5'-flanking DNA, 2.8 kb of genomic DNA upstream from exon 1 was sequenced (Fig. 2). The results were analyzed using a computer database in the program SIGNAL, which compared this sequence with known eukaryotic cis-elements. Several putative cis-elements, which have been shown to be important in the expression of other epidermal genes were identified (Fig. 2). These regulatory cis-elements included four AP-1, three AP-2, and one SP-1 binding site (15, 16). In addition, a putative glucocorticoid-responsive element (TGTCCT) (17) was found in positions −2205 to −2200 (in relation to the translation initiation site). A CK-8mer, a sequence suggested to confer keratinocyte-specific expression to certain keratin genes was identified in positions −2395 to −2388 (18). Three segments conforming to the consensus sequence, T(G/T)NNNG(C/T)AA(G/T), for C/EBP cis-element, which has been implicated in terminal differentiation processes (19, 20), were identified. Finally, an Oct-like motif, ATGCAAGC (at −190 to −182), was identified. The functionality of these putative cis-elements will be determined in future studies. No canonical TATA or CAAT sequence was identified within the first 300 bp upstream from the translation initiation site. However, a 17-bp region at −154 to −137 upstream from the translation initiation site was found to be GC-rich (82.3%).

Demonstration of PVA mRNA Expression in Cultured Keratinocytes—Northern analysis of keratinocyte mRNA isolated from cells grown under conditions described under “Materials and Methods” revealed the expression of specific transcripts for the bullous pemphigoid antigen-1 (BPAG1) and bullous pemphigoid antigen-2 (BPAG2) genes of 9.0 and 6.0 kb, respectively (21). Hybridization of the same filters with the 468-bp cDNA revealed the presence of characteristic 6.0-kb PVA mRNA (Fig. 3), indicating that the NHK cells used in this study express the PVA gene.

Identification of Transcription Initiation Site(s)—To determine the transcription initiation site(s) within the 5'-flanking DNA, primer extension utilizing human keratinocyte RNA as a template was performed. A major primer extension product with an apparent size of 112 nucleotides was detected in two separate experiments. This extension product would place the transcription initiation site(s) approximately 140 to 137 bp upstream from the translation initiation site (Fig. 4). Extended exposure of the autoradiograms revealed two minor bands, approximately 143 and 91 bp in size (see Fig. 4). The transcription initiation sites for these two extension products correspond to positions of about −140 and −88, respectively.

To confirm the results of the primer extension experiments, an RT-PCR analysis of PVA mRNA was performed (Fig. 5). For
this purpose, the first strand cDNA was generated using an antisense oligonucleotide, D3 (Fig. 5), which extended the reaction from 529 to 509 toward the 5'-end of the mRNA. The newly synthesized cDNA was then used as template for PCR amplification using a downstream primer (D1, see Fig. 5) and a set of upstream primers (numbers 1–8, and a), which correspond to different segments of the genomic DNA. In parallel, PCR amplification of genomic DNA using the same set of upstream primers and a downstream primer in intron 1 (D2 in Fig. 5) was performed. PCR analysis of cDNA showed that primer pairs containing the upstream primers 1–4 clearly resulted in PCR products of the expected size, indicating that these sequences were present in the cDNA synthesized from the keratinocyte mRNA (Fig. 5C). In contrast, primer pairs containing the upstream primer 5, 6, 7, or 8 failed to amplify the cDNA, indicating that these sequences were not present in the cDNA. An additional primer, a, was then designed to define the sequence between primers 4 and 5, and was used for similar PCR analysis. The primer a did not yield a product when cDNA was used as a template (data not shown). When genomic DNA was used as a template, primers 1–8 (Fig. 5C) and a (not shown) resulted in PCR products of the expected sizes. These data narrow the region of the transcription initiation site to the sequences between primers 4 and a and are consistent with the site at about –140, thus confirming the primer extension analyses. Collectively, the primer extension and RT-PCR analyses indicated that there are three potential transcription initiation sites for the PVA gene.
Further downstream 5'-deletion constructs revealed evidence for cell type-specific expression of the promoter. Specifically, while transfusion within constructs extending from −300 to −200 showed predominant expression in keratinocytes, transfusions with the construct −160 phPVA0.16 revealed −2-fold higher CAT activity in fibroblasts than in keratinocytes (Fig. 6). These results suggested the existence of keratinocyte-specific cis-elements between −200 and −160.

To fine map the putative cis-elements conferring keratinocyte-specific expression to the PVA gene, 30-bp fragments covering 120 bp between −290 and −170 of the promoter were cloned in front of a TK-CAT construct and used for similar transfection in keratinocyte and fibroblast cultures. The results, shown in Fig. 7, revealed that the relative activity was markedly higher in keratinocytes with a construct containing the segment from −200 to −170 in front of the TK promoter. Careful examination of the corresponding sequence revealed the presence of the ATGCAAGC motif, which has partial homology to the Oct consensus sequence, ATGCAAT (Fig. 7). However, development of TK-CAT constructs containing the −200 to −170 segment, in which 2-bp nucleotide substitutions in the putative Oct domain were introduced, revealed that this motif was not functional in providing keratinocyte-specific expression.

**DISCUSSION**

The cadherins constitute a superfamilly of cell adhesion proteins, which can be further divided into two subfamilies, namely the classical cadherins and desmosomal cadherins (10). Within the desmosomal cadherins, there are two classes of adhesion molecules, the desmogleins and the desmocollins (25).

In this study, we cloned and analyzed the gene for pemphigus vulgaris antigen, a desmoglein type cell adhesion molecule. Specifically, we have characterized the human PVA gene, DSG3, and the upstream regulatory sequences. The entire human DSG3 gene was found to consist of 15 exons, and the gene has been mapped to human chromosome 18q (26).

To examine the evolutionary divergence of the gene structure between different cadherins, the sizes of the DSG3 exons were compared with those in previously published cadherin genes (Table II). The exon sizes, in general, demonstrated striking conservation in exons 3–11 between human DSG3, bovine DSG1, and mouse P cadherin (Table II). In particular, the conservation of exon sizes between DSG1 and DSG3 was remarkable, with the exception of exon 12. Careful alignment of the genes encoding classical cadherins of different species with the desmoglein genes indicated that the mouse E-cadherin, the human and mouse N-cadherin, and the chicken L-cadherin genes consisted of 16 exons, the correspondence of DSG3 exons 3–11 being highest with cadherin gene exons 4–12 (Table II). Thus, the cadherin genes may have an evolutionary insertion of a 5'-exon, or alternatively, DSG1, DSG3, and P-cadherin genes may have an evolutionary deletion of a 5'-exon (Table II).

In contrast to the conservation of the exon sizes, the introns varied widely in their sizes (22, 27–31). It has been suggested that the variation in the intron size prevents intergenic crossover events within the cadherin gene family (29). The exact size of the first intron of DSG3 could not be determined, since exon 1 and exon 2 resided in different phaseed clones, which were apparently not overlapping, as judged by the lack of common restriction enzyme fragments. Attempts to PCR amplify intron 1 sequences from genomic DNA were unsuccessful. However, the sequence downstream from exon 1 in clone 1 was 2 kb in size, and the sequence upstream from exon 2 in clone 3 was 4.5 kb in size. Thus, the first intron appears to be at least 6.5 kb in size. This observation is consistent with the exon-intron organization of the 5'-end of other cadherin genes, which also
demonstrate the presence of large first introns, up to 100 kb in size (28–30, 32). Thus, the 5' end of the human PVA promoter gene has similarities with other previously characterized non-human cadherins.

Genomic cloning of the PVA sequences allowed identification of three transcription initiation sites, with an apparent major site residing at the approximate position of 2109 and two minor transcription initiation sites at positions 2140 and 288, in relation to the putative translation initiation site. These investigators assigned the first methionine residue as the most amino-terminal amino acid of the putative polypeptide. We have recently cloned the corresponding mouse cDNA sequences (33) and demonstrated that the first methionine residue within the human sequence is conserved in the mouse gene, while the second methionine residue is substituted by a threonine. Thus, the first methionine residue in the human sequence is also likely to be the first amino acid of the putative polypeptide and is consequently assigned as +1.

Examination of the upstream sequences identified features characteristic of eukaryotic promoters, including previously cloned mammalian cadherin promoters (22–24). Specifically, the PVA promoter had a GC-rich region around 2155 to 2137 upstream from the translation initiation site. Furthermore, similar to mouse E-cadherin and P-cadherin, as well as chicken L-cadherin sequences, there was no canonical TATA box. These features are consistent with identification of multiple transcription initiation sites within the promoter region (34).

Pemphigus vulgaris antigen has previously been shown to be expressed primarily in stratifying squamous epithelia, such as the epidermis of the skin. In this study, we developed a series of functional PVA promoter/CAT reporter gene constructs and tested their tissue-specific expression by parallel transfections of cultured keratinocytes and fibroblasts. The results clearly demonstrated that the PVA promoter is active in normal human keratinocytes, while fibroblasts expressed the promoter activity at an extremely low level. These observations indicate that the 5'-flanking sequences of the PVA gene contain cis-elements that confer tissue-specific expression to the gene. This observation is also consistent with the presence of tissue-specific cis-elements found in the 5'-flanking regions of other epidermal specific genes, such as BPAG1, and keratins 5 and 14 (14, 35). This does not, however, exclude the role of possible regulatory elements located within introns or the 3'-flanking DNA in tissue-specific expression. For example, the keratin 1 gene contains a negative tissue-specific element in the 3'-flanking DNA (36).

Transient transfections of 5'-deletion constructs of the PVA

![Fig. 6. Development of human PVA promoter/CAT constructs and their relative activities.](image)

![Fig. 7. Identification of a keratinocyte-specific segment within the PVA promoter.](image)
gene suggested that the tissue-specific elements reside within 0.3 kb of the 5'-upstream sequences. Careful examination of putative cis-elements within this region demonstrated that there is an AP2 site at -149 to -142. This site is at the border of the most upstream transcription initiation site, and it is conceivable that this AP2 element plays a role in keratinocyte-specific expression of the PVA gene. This suggestion is supported by previous findings on the role of AP2 in expression of various keratins, including KRT5 and KRT14 (16). Search for other putative cis-elements in the PVA promoter region identified several AP1 binding sites, two additional AP2 sites, a putative glucocorticoid-responsive element, and one Sp1 site, but the functional significance of these cis-elements is currently unknown. It was of interest that a sequence previously designated as CK-8mer (18), which has been suggested to confer tissue-specific expression to human keratin14, was found in the position -2,395 to -2,388. It should be noted that a similar CK-8mer sequence has previously been detected in the 230-kDa bullous pemphigoid antigen gene promoter region without apparent functional significance (14). It appears, therefore, that this particular sequence may not play a significant role in conferring keratinocyte-specific expression to the PVA gene. Instead, transient transfections with the 5'-deletion constructs of the PVA promoter used in this study were able to identify a region extending from -200 to -170 that conferred keratinocyte-specific expression either when tested in the context of either its own promoter or a heterologous TK promoter linked to the CAT reporter gene. This segment was found to contain a sequence homologous to the Oct motif (37, 38). The Oct transcription factors belong to the POU domain family of proteins known to function as developmental regulators, both in early embryogenesis and in cell type-specific terminal differentiation events (37, 38). Three of these factors, Skn-1a, Oct-1, and Oct-4, have been shown to be enriched within the epidermis, suggesting a role in the keratinocyte differentiation process. Thus, the putative Oct cis-element identified within the proximal region of the PVA promoter was suggested to contribute to the epidermal specific expression of this gene. However, nucleotide substitutions in this motif disproved this suggestion. Thus, dynamic interplay between a repertoire of distinct regulatory elements is likely to be required for proper tissue and differentiation-specific expression of the PVA gene (39).

In summary, the results of this study, which characterize the exon-intron organization of the human PVA gene and demonstrate tissue-specific expression of the gene at the transcriptional level, provide novel insight into understanding the normal physiology of human epidermis.

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| Exon No. | DSG3 human | DSG1 bovine | P-cadherin mouse | E-cadherin mouse | N-cadherin human | N-cadherin mouse | L-cadherin chicken |
|----------|------------|-------------|------------------|------------------|-----------------|-----------------|------------------|
| 1        | 184        | ND          | 1000             | 175              | ND              | 112             | 112              |
| 2        | 33         | 35          | 74               | 121              | ND              | 112             | 115              |
| 3        | 88         | 88          | 126              | 224              | 227             | 227             | 230              |
| 4        | 154        | 156         | 156              | 147              | 147             | 135             |                  |
| 5        | 146        | 144         | 145              | 156              | 156             | 156             |                  |
| 6        | 168        | 166         | 176              | 145              | 145             | 145             |                  |
| 7        | 129        | 136         | 176              | 176              | 173             | 174             | 176              |
| 8        | 186        | 186         | 186              | 129              | 138             | 129             |                  |
| 9        | 270        | 261         | 241              | 183              | 186             | 186             |                  |
| 10       | 140        | 140         | 146              | 245              | 254             | 254             | 245              |
| 11       | 225        | 291         | 225              | 146              | 143             | 143             | 143              |
| 12       | 401        | 134         | 210              | 225              | 234             | 234             | 225              |
| 13       | 63         | 73          | 210              | 225              | 234             | 234             | 222              |
| 14       | 283        | 209         | 147              | 131              | 131             | 131             |                  |
| 15       | 864        | 1017        | 833              | 144              | 165             | 165             | 144              |
| 16       |            |             | 1841             | 1391             | 1481            | >1000           |                  |

The sizes of the exons (bp) of DSG3 were compared with published cadherin genes. The reference sources are in parentheses. ND, not determined.
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