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Review article

Novel isoforms of transcript of the *EDA* gene confirm X-linked inheritance of anhidrotic ectodermal dysplasia

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Abstract. This review is focused on recent developments of the genetic studies on anhidrotic ectodermal dysplasia (EDA) reported since the publication of our previous review in 1997. In addition to cloning of the mouse homologue of the *EDA* gene, the Tabby gene, recent discoveries of the novel transcript isoforms by several research groups as well as by our group, were presented and discussed in the context of the mode of inheritance of anhidrotic ectodermal dysplasia. The paradox that despite typical phenotype, no mutations were evidenced in the originally described two-exon isoform of the *EDA* gene, was explained. It was also pointed out that despite careful analysis of the structure of the entire gene and its regulatory region, no mutations were found in some of the patients, suggesting the existence of autosomal dominant or recessive forms of the disease, which requires further investigations.

Key words: alternatively spliced forms, anhidrotic ectodermal dysplasia, Christ-Siemens-Touraine syndrome, *EDA* gene, gene transcript, *Tabby* gene.

Introduction

Anhidrotic ectodermal dysplasia (EDA), known also as the Christ-Siemens-Touraine syndrome (MIM 305100; McKUSICK 1998), is an X-linked recessive disorder resulting from abnormalities of ectodermal-mesodermal interaction during embryonic development (KERE et al. 1996). However, autosomal dominant and recessive forms should also be considered (JORGENSON et al. 1987, HO et al. 1998, MUNOZ et al. 1998, BAALA 1999, HEADON, OVERBEEK 1999, MONREAL et al. 1999). Most of the patients with the diagnosis of EDA show sev-

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eral characteristic symptoms: deficiency (*oligodontia*) or lack of teeth (*anodontia*), overheating (*hyperthermia*), which results from diminished sweating (*hypohidrosis*), and sparse hair (*hypotrichosis*) (McKUSICK 1998).

A candidate for the *EDA* gene was identified by KERE et al. (1996). This gene contains two exons, encoding a 135 amino acid protein, but the function of the protein product and its role in the development of skin appendages remains unknown. Two years ago we reviewed genetic mapping and cloning of the *EDA* gene (KOBIELAK et al. 1997). However, we pointed out that the principal question whether or not the *EDA* gene was responsible for the symptoms of anhidrotic ectodermal dysplasia had not been answered, since no explanation had been provided why so few mutations in the *EDA* gene had been detected. We postulated that the originally described gene was responsible for the symptoms of EDA and might contain additional yet unidentified exons that harbour the mutations (KOBIELAK et al. 1997)

The purpose of this review is to further review and discuss recent literature concerning the genetic and molecular background of anhidrotic ectodermal dysplasia and to present possible implications of the laboratory findings in understanding the process of development of skin appendages. The problem is important since it is closely related to differentiation of skin appendages during embryonic life and might provide a useful model for studies of the development of specialised cells.

Mutation in the regulatory region of the *EDA* gene

Only 9 mutations were found in the two exons of the *EDA* gene in 118 patients described in literature (KERE et al. 1996). Moreover, the direct sequencing of the entire gene revealed no mutations in our group of 18 patients (KOBIELAK et al., unpublished data). Therefore we assumed that if the *EDA* gene contained only two exons, the defects would reside in the regulatory region.

There were two reasons to believe that this was the right assumption. Firstly, the hair-specific motif HK-1 (CTTTGAAGA) was identified, 420 bp upstream of the transcription start site (KERE et al. 1996). This site is a target motif (ROGERS, POWELL 1993) for the lymphoid enhancer-binding factor-1 (LEF-1), involved in differentiation of keratinocytes (TRAVIS et al. 1991, ZHOU et al. 1995). Secondly, other investigators demonstrated hair follicle and teeth abnormalities, resembling those of EDA patients, in knockout mice devoid of the *Lef-1* gene (van GENDEREN et al. 1994, ZHOU et al. 1995). Since localisation of the *LEF-1* gene on chromosome 4 (MILATOVICH et al. 1991) excluded X-linked inheritance, we decided to search for mutations in the regulatory region. Analysis of the regulatory region in our group of 18 patients revealed in one of the patients an addition of a single nucleotide 32 bp upstream from the HK-1 motif (KOBIELAK et al. 1998). However, to date there is no evidence that this mutation impairs expression of the *EDA* gene.

Cloning of the *Tabby* gene

The role of the *EDA* gene in the process of differentiation of skin appendages was confirmed by the investigations in mice hemizygous for the *Tabby* gene (BLECHER 1986). Those animals exhibited similar phenotypic features to those found in EDA patients, and this might provide an experimental model of the disease. Moreover, the localisation of the mouse *Tabby* gene on the X chromosome corresponds to the position of the *EDA* gene in humans (BROCKDORFF et al. 1990, ZONANA et al. 1993).

To clone the *Tabby* gene, hybridisation probe corresponding to exon 1 of the *EDA* gene was used to screen the mouse cDNA library, resulting in identification of three alternatively spliced *Tabby* gene transcripts (Figure 1).

The longest isoform (Figure 1A), that encodes a 391-aa transmembrane protein, ectodysplasin-A (FERGUSON et al. 1997), was concomitantly described by another research group (SRIVASTAVA et al. 1997).

The shortest of the isoforms (Figure 1B), in addition to exon 1 contains exon 3 (designated 3a), extended by 135 nucleotides, corresponding to a 177aa protein product. In the one-exon intermediate form of the transcript (Figure 1C) exon 1 was extended by 264 nucleotides and encodes a 220aa protein.

The sequence of exon 1 of the *Tabby* gene exhibited 87% homology to the sequence of the same exon of the *EDA* gene. However, no sequence homologous to

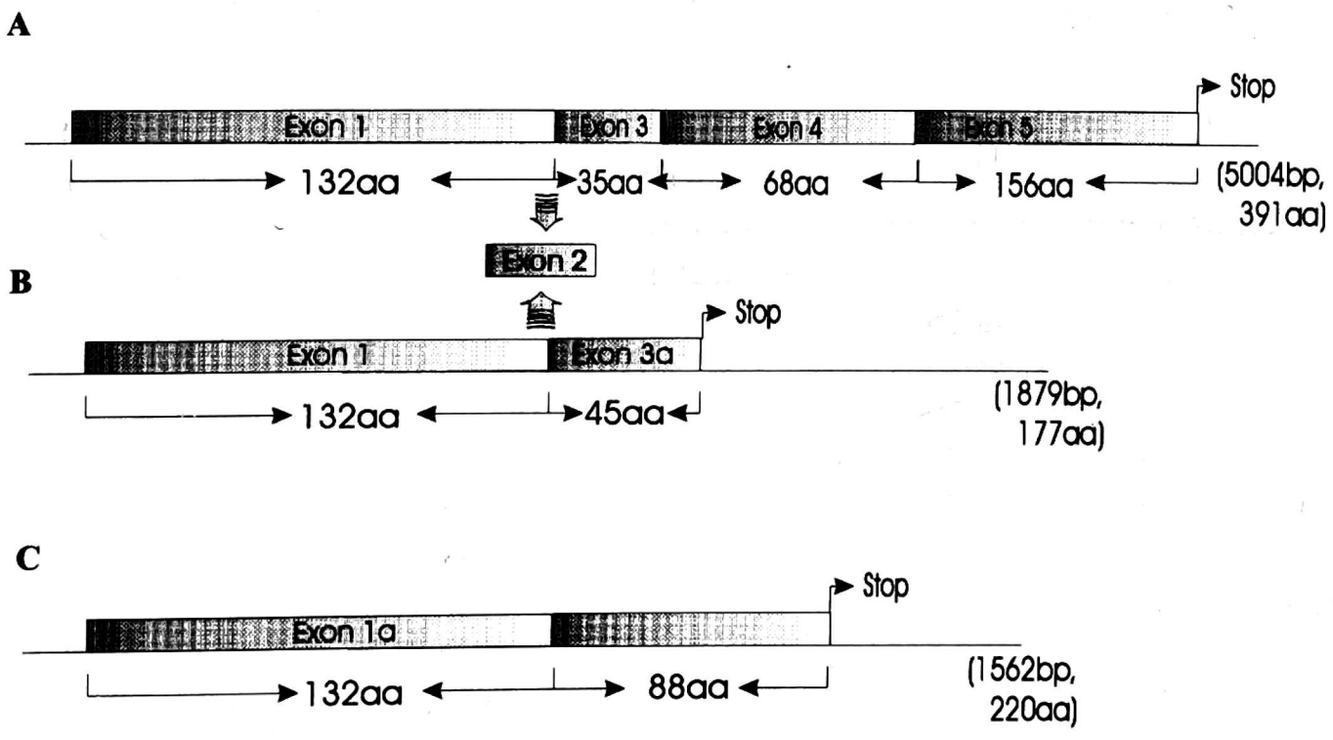

Figure 1. Isoforms of transcript of the *Tabby* gene

The size of the transcripts and the length of the amino acid chains are shown in round brackets. The numbers below each box represent amino acids encoded by the respective exons. Letters A, B and C signify appropriate transcript isoforms. Arrows indicate lack of exon 2. The termination codons are marked as Stop.

exon 2 of the *EDA* gene has been evidenced in all isoforms of the mouse transcripts (Figure 1). Moreover, the sequences of the regulatory regions of both genes showed 96% homology, with a consensus sequence around the HK-1 motif, a putative binding site of the transcription factor LEF-1.

Novel isoforms of the *EDA* gene transcript

The predicted longest protein product of the mouse transcript (FERGUSON et al. 1997, SRIVASTAVA et al. 1997) was encoded by three more exons than the protein product of the *EDA* gene from sweat glands (KERE et al. 1996). This suggested the possibility that the *EDA* gene contains novel exons that might be removed by alternative splicing and therefore were not detected in the transcript originally described (Figure 2). Investigations conducted independently in two laboratories revealed an isoform of the *EDA* gene transcript (Figure 2A) homologous to the longest isoform of the *Tabby* gene (MONREAL et al. 1998, BAYES et al. 1998). This isoform (Figure 2A), encoding a 391aa protein, exhibited 94% homology to its mouse equivalent (Ta protein), although the number of exons in the gene transcript was different (MONREAL et al. 1998).

At the same time six additional isoforms of the *EDA* gene transcript have been discovered (BAYES et al. 1998), confirming that the gene transcript undergoes alternative splicing (Figure 2A-F). All of these isoforms contain exon 1a and a modified form of this exon (1b, 1c, 1d or 1e), as in the case of isoforms C, F, D and E, or either exon 3a (in isoform B) or exon 2a (in isoform F). However, the mechanism of tissue-specific splicing leading to these isoforms has not been investigated and the role of protein products of these differentially spliced isoforms has not been elucidated.

Mutations in the newly discovered exons of the *EDA* gene

The discovery of the novel exons has prompted the search for mutations in these exons. As expected, mutations have been found in most of the newly discovered exons downstream from exon 2. This resulted in the rise in the detection rate from approximately 7% to about 95%. To date, 47 mutations have been described in literature in 7 exons of the *EDA* gene in 44 patients (Table 1). The results from literature (KERE et al. 1996, BAYES et al. 1998, FERGUSON et al. 1998, MONREAL et al. 1998) revealed that the majority of the mutations (mostly substitutions of a single nucleotide) were localised in exon 1, with a smaller number detected in exons 5, 8, 9 and 3. In exons 8, 9 and 3 mostly substitutions of a single nucleotide were identified, whereas in exon 5 mostly deletions from 18 bp to 36 bp were found in the region, encoding the motif Gly-X-Y, homologous to the helical domain of collagen. No mutations were detected in exons 4 and 6, and only one in exon 7 (Ta-

ble 1). However, the cDNAs obtained from the patients carrying these mutations have not been cloned and expressed to demonstrate the effect of the mutation on the function of the protein product of the gene.

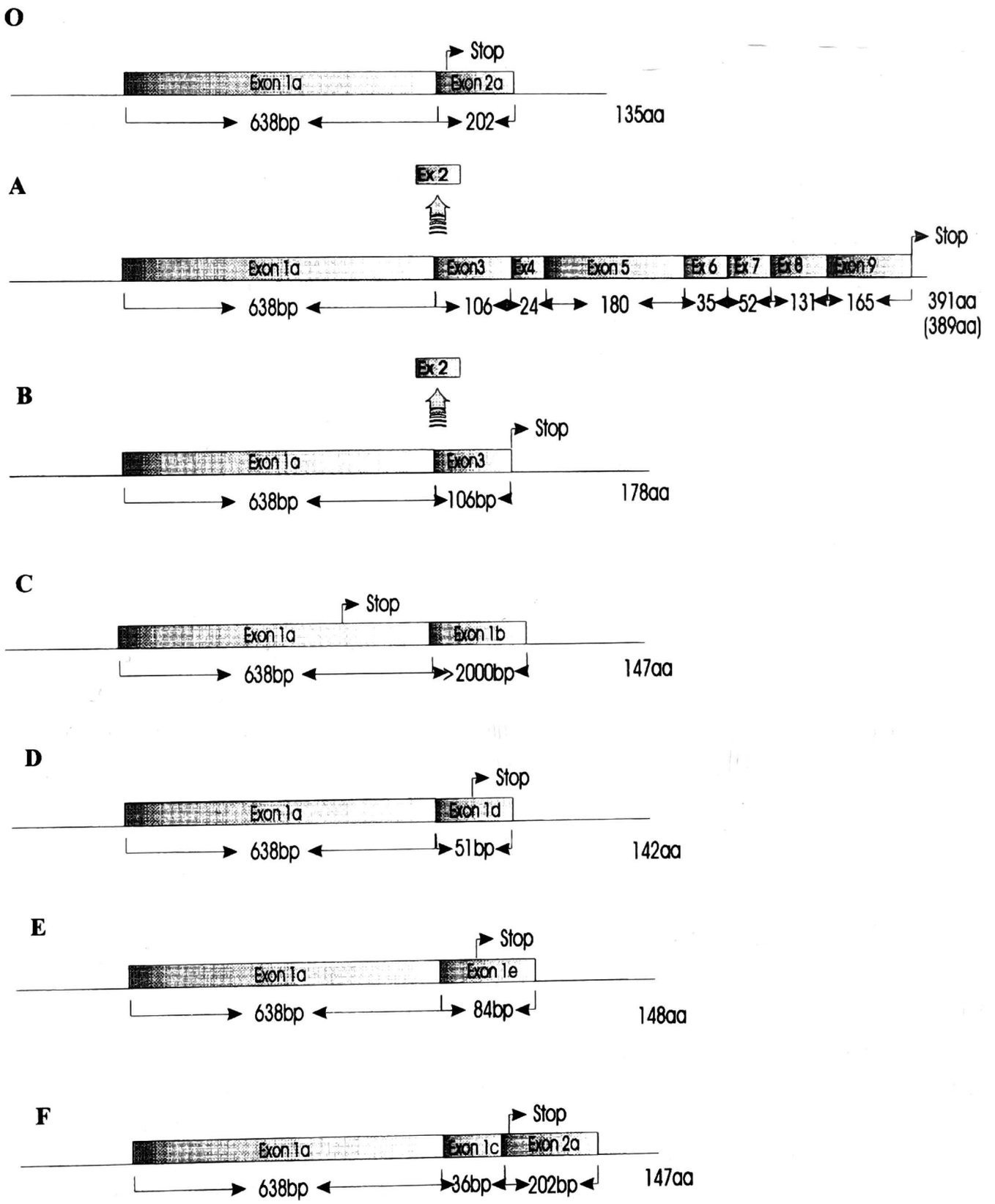

Figure 2. Isoforms of transcript of the *EDA* gene

Letters: O, A, B, C, D, E and F signify respective transcript isoforms. The difference in the amino acid number of isoform A variant is given in brackets. The other symbols as in caption to Figure 1.

Table 1. List of the mutations in the exons of the *EDA* gene described to date

No.	Family number	Nucleotide change	Exon	References
1	EDA19	G180A; T423C	1	KERE et al. 1996
2	EDA1075	G187T	1	FERGUSON et al. 1998
3	EDA7	G187T	1	KERE et al. 1996
4	EDA1166	287insC	1	KERE et al. 1996
5	EDA12	363insC	1	KERE et al. 1996
6	EDA1180	C366T	1	FERGUSON et al. 1998
7	EDA-D14	427delT	1	FERGUSON et al. 1998
8	EDA1129	G429A	1	FERGUSON et al. 1998
9	EDA73	G448T	1	KERE et al. 1996
10	EDA1113	G448T	1	FERGUSON et al. 1998
11	EDA52	494delT	1	KERE et al. 1996
12	EDA1024	494delT	1	KERE et al. 1996
13	EDA1108	494delT	1	FERGUSON et al. 1998
14	EDA46	594-595delCC; A593G	1	FERGUSON et al. 1998
15	EDA1013	C636T; G227A	1	KERE et al. 1996
16	EDA13	302insGGGAGCGA	1	BAYES et al. 1998
17	EDA4	C708T	3	BAYES et al. 1998
18	EDA9	ekson 3 del	3	BAYES et al. 1998
19	EDA1081	C704T	3	MONREAL et al. 1998
20	EDA1095	C707T	3	MONREAL et al. 1998
21	EDA1039	G708A	3	MONREAL et al. 1998
22	EDA11	AIVS5-2G	IVS5	BAYES et al. 1998
23	EDA1011	C867T	5	MONREAL et al. 1998
24	EDA1019	G912C	5	MONREAL et al. 1998
25	EDA1050	794-829 del	5	MONREAL et al. 1998
26	EDA1204	794-829 del	5	MONREAL et al. 1998
27	EDA1018	803-830 del	5	MONREAL et al. 1998
28	EDA1097	904-938 del	5	MONREAL et al. 1998
29	EDA3	836-841insC	5	BAYES et al. 1998
30	EDA10	801/814 del 18	5	BAYES et al. 1998
31	EDA2	891/901 del 18	5	BAYES et al. 1998
32	EDA15	789/795 del 36	5	BAYES et al. 1998
33	EDA1197	A986T	7	MONREAL et al. 1998
34	EDA6	G1113A	8	BAYES et al. 1998
35	EDA5	G1113T	8	BAYES et al. 1998
36	EDA7	G1134C	8	BAYES et al. 1998
37	EDA14	G1137A	8	BAYES et al. 1998
38	EDA1007	G1136A	8	MONREAL et al. 1998
39	EDA1002	G1136A	8	MONREAL et al. 1998
40	EDA1001	G1202T	9	MONREAL et al. 1998
41	EDA1021	G1285A	9	MONREAL et al. 1998
42	EDA1126	G1285A	9	MONREAL et al. 1998
43	EDA1073	C1308A	9	MONREAL et al. 1998
44	EDA1022	G1311C	9	MONREAL et al. 1998

EDA – anhidrotic ectodermal dysplasia; del – deletion; ins – insertion; IVS5 – splice site alteration in intron 4.

Protein product of the *EDA* gene

The initially described two-exon transcript isoform of the *EDA* gene (Figure 2 isoform O) encodes a 135aa protein product, belonging to class II transmembrane receptors that have the N-terminal domain located within the cytosol, and the C-terminal domain projecting outward (KERE et al. 1996).

The protein product of the longest isoform of the *EDA* gene shows high homology to the protein product of isoform-O of the *EDA* gene. Both a single transmembrane domain and an intracellular N-terminal domain are present in the two isoforms, as well as in the Ta protein. However, the C-terminal hydrophylic domain reveals homology only to the largest protein product of the human and the mouse isoforms of the transcript. Moreover, the predicted C-terminus projecting outward (EZER et al. 1997) includes 19 repeats (Gly-X-Y) which may form a triple helix stabilised by three sulphate bridges.

The fact that in 95% of EDA patients the mutations reside within the longest isoform of the *EDA* gene suggests a critical role of this form of transcript in the development of skin appendages.

Concluding remarks

The question whether or not the *EDA* gene is responsible for the symptoms of anhidrotic ectodermal dysplasia can now be answered. The discovery of novel exons enabled to increase the detection rate of the mutation to over 95% in the patients who did not exhibit any changes in the two-exon isoform of the *EDA* gene transcript. However, in some patients with the symptoms of anhidrotic ectodermal dysplasia no mutations were detected in the entire *EDA* gene.

These observations, combined with analysis of the pedigrees of the patients, suggested that there might exist different type(s) of inheritance of the disease. Some cases could be diagnosed as an autosomal recessive or autosomal dominant, clinically indistinguishable from the X-linked form (JORGENSON et al. 1987, MUNOZ et al. 1998). Moreover, further investigations allowed mapping of the genes responsible for both recessive and dominant forms of anhidrotic ectodermal dysplasia to chromosome 2q11-q13 (HO et al. 1998, BAALA 1999). Recently, strong evidence for the existence of two other genes (*crinkled* and *downless*) responsible for autosomal recessive and dominant forms of the disease was presented (HEADON, OVERBEEK 1999, MONREAL et al. 1999).

Although many isoforms of the *EDA* gene transcript have been discovered, their function during embryonic development has not been elucidated. This provides new directions for future investigations.

The question of the role of the protein product of the *EDA* gene in the differentiation of skin appendages still remains unanswered. Although the structure and

localisation of this protein in cell membrane have been determined, its function is still unknown. This protein, which belongs to type II receptors, might interact with a yet unidentified ligand and might play a critical role in cellular signalling as well as cell adhesion and/or migration. This hypothesis, however, needs to be verified.

Anhidrotic ectodermal dysplasia provides not only an excellent model to investigate the differentiation of skin appendages but also constitutes a suitable model to study signal transduction within the differentiating cell. These yet unsolved problems indicate the prospects for future research in this field.

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