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Characterization of dystrophic epidermolysis bullosa patients for collagen VII therapy

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Dystrophic EB (DEB) is a blistering skin disease caused by mutations in the gene (*COL7A1*) encoding type VII collagen (C7). DEB can be inherited by either dominant (DDEB) or recessive (RDEB) mechanisms. RDEB results in severe wounds and scarring, as well as extracutaneous manifestations such as esophageal strictures, chronic anemia, and pseudosyndactyly. DDEB is generally a milder form, with fewer and less severe blisters.(1)

COL7A1 contains 118 exons. The central Gly-X-Y region is flanked by two non-collagenous regions: the amino-terminal NC1 domain (exons 1-28) and the carboxy-terminal NC2 domain (exons 112 – 118). RDEB patients may either express the NC1 amino-terminal fragment of C7 (NC1[+]) or lack it (NC1[-]).(2) The NC1 region has been reported to contain the major antigenic epitopes of C7, although immune responses to other C7 regions have been noted.(3) In the autoimmune disease epidermolysis bullosa acquisita (EBA), IgG autoantibodies bind to various epitopes in C7, causing dermal-epidermal separation and blistering.(3)

As a screening tool for C7 replacement trials, we investigated whether NC1 expression can be predicted using genetic testing.(2, 4) We proposed that subjects who do not express the NC1 domain (NC1[-]) could be predicted by two loss of function mutations in exons 1-28. We compared genetic testing data with indirect immunofluorescence (IIF) using LH7.2 antibody and Western blot techniques using FNC1 antibody (Figure 1). Genetic testing predictions correlated with Western blot results for 21/21 (100%) samples (Tables 1, S1). A total of 39 mutations were identified including nine previously unreported mutations (Table S1).

IIF staining with LH7.2 displayed significant variability, correlating with FNC1 Western for 11/22 (50%) samples tested (Tables 1, S1). LH7.2 binds to an epitope between amino acids 36 (exon 2) and 153 (exon 4); the NC1 domain spans up to exon 28.(5) Therefore, a sample may show positive LH7.2 staining even if the full NC1 domain is not produced, as seen in subjects 15 and 17. These results illustrate the variability in using IIF with LH7.2 as a diagnostic tool for NC1 expression.

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Revertant mosaicism is another confounder to biopsy analysis.(6) Skin biopsies for EB diagnosis are obtained from uninjured, unscarred skin, which can be difficult to locate in older EB patients, especially those with RDEB. It is possible that the sites selected for biopsy may actually be areas of undiagnosed revertant mosaicism, which would be positive for NC1 on IIF and Western blot but predicted to be NC1[–] by genetic testing.

Circulating antibodies to C7 were detected using two ELISA assays. By the Stanford ELISA (Supplementary Methods) 7/26 had antibodies and 11/26 had antibodies by the commercial ELISA (MBL International, Woburn, MA). Only three subjects were positive by both assays (Table 2). Most notably, all subjects were negative for IgG, IgM, IgA or complement deposits at the dermal-epidermal junction by direct immunofluorescence (DIF) on their own skin, providing evidence that these antibodies are not pathogenic, as has been previously reported by other groups.(7–9)

Nine subjects had previously received synthetic allografts, which would expose the recipient to C7, although there have been no previous reports of immune response.(10) Only one subject (#18) was positive for C7 antibodies and had previously received allograft transplants (Table 2). One subject (#17) had received allografts but was not able to give a blood sample; therefore testing for C7 antibodies was not performed. This subject did not have any cross-reactivity to the basement membrane by immune complexes on DIF. We did not find an association between previous allograft therapy and antibody production. The absence of antibodies in the majority of subjects exposed to full length C7 is encouraging for future C7 therapies.

Many C7 replacement therapies for DEB are currently in development. This study focuses on important characteristics to identify candidates who are at the least risk of having adverse autoimmune reactions to the C7 therapy. We believe that the first subjects should be NC1[+], determined by analysis of genetic testing. Subjects with two loss of function mutations between exons 1-28 are expected to be NC1[–] and would be excluded. The exception would be subjects with revertant mosaicism who may already express functional C7 but have two loss of function mutations between exons 1-28. Expression of non-tissue binding C7 autoantibodies is less important in subject selection as they may not be pathogenic. However, in early studies, subjects should be negative for immunoglobulin or complement deposits at the dermal-epidermal junction, by DIF. Both DIF and genetic testing are standardized laboratory tests. During the treatment protocol, it will be important to carefully monitor subjects for immune responses to therapies. Ultimately, the success of these future clinical trials will depend upon appropriate subject selection, which should minimize adverse responses to therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Representative image of Western blot, showing full length C7 expression (#5), NC1 expression (#6), and complete absence of C7 (#16).

NC1 expression, LH7.2 expression, and genetic mutations in RDEB subjects whose cells were cultured. Sorted by NC1 result and then actual IIF result.

Table 1

	ID	Predicted NC1	Actual NC1	Predicted IIF (LH 7.2)	Actual IIF (LH 7.2)	Mutation 1 Location:	Mutation 2 Location:
NC1[+]	1	+	+	±	+	E 84	UNK
	7	+	+	±	+	E 3	E 68
	8	+	+	±	+	E 3	E 63
	10	+	+	±	+	E 84	E 65/I 64
	12	+	+	±	+	E 50	E 74
	2	+	+	±	±	E 14	E 117
	4	+	+	±	±	E 3	E 86
	5	+	+	±	±	E 19	E 80
	9	+	+	±	±	E 79	E 93
	13	+	+	±	±	E 52	I 36
	3	+	+	±	-	E 2	E 54
	6	+	+	±	-	E 45	E 45
NC1[-]	11	+	+	ND	ND	E 14	E 54
	15	-	-	-	±	E 3	E 15
	17	-	-	-	±	I 12	I 26
	14	-	-	-	-	E 6	E 6
	16	-	-	-	-	E 4	E 15

E = Exon. I = Intron. UNK = Unknown. ND = Not done.

Table 2

C7 antibodies, sorted by NC1 result, then Stanford ELISA result

ID	NC1	Stanford ELISA	MBL kit	DIF (C3, IgG, IgM, IgA)	Prev. Graft Info	Time between graft and study
1	+	-	1.69	-		
2	+	-	1.19	-		
3	+	-	34.27	-		
4	+	-	3.90	-	Apligraf	3 yrs.
6	+	-	1.52	-	Dermagraft	> 1 yr.
22	+	-	1.07	-		
8	+	-	7.96	-	Apligraf	10 yrs.
9	+	-	16.94	-	Apligraf	2 yrs.
23	+	-	0.62	-		
24	+	-	4.46	-		
12	+	-	48.84	-		
5	+	+	19.93	-		
7	+	+	1.07	-		
10	+	+	5.42	-		
11	+	+	22.25	ND		
25	+	+	0.51	-		
13	+	ND	34.33	-	Apligraf	9 yrs.
14	-	-	2.15	-	Apligraf	10–13 yrs.
15	-	-	15.08	-		
16	-	-	5.65	-	Apligraf	6 mos.
17	-	ND	ND	-	Apligraf	7 yrs.
19	UC	-	17.05	-	Apligraf	> 1 yr.
20	UC	-	8.36	-		
26	UC	-	1.47	-		
18	UC	+	ND	-	Apligraf	> 1 yr.
21	UC	+	14.34	ND		

KC grafts = previous grafting with keratinocyte allografts. UC = Unable to culture. ND = Not done. Note that values above 7 for the MBL kit indicate C7 antibodies.