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## Recessive dystrophic epidermolysis bullosa: the origin of the c.6527insC mutation in the Spanish population

MADAM, Recessive dystrophic epidermolysis bullosa (RDEB; OMIM #226600) is a rare congenital mechanobullous disease.<sup>1</sup> To date, about 550 distinct mutations in the COL7A1 gene (OMIM \*120120) have been identified as responsible for this pathology. Highly recurrent mutations are seldom associated with RDEB<sup>2</sup> (<http://www.deb-central.org/>). Interestingly, the most prevalent COL7A1 mutation causing RDEB in Spain, c.6527insC, exhibits an unusually high recurrence (46.3% of alleles) being prevalent among patients from the southern half of the Iberian peninsula.<sup>3</sup> We have previously suggested a founder effect for c.6527insC as Spanish patients carrying this mutation share an intragenic ancestral haplotype (H5) of approximately 30 kb, which is present at a low frequency in the general, ethnically matched population.<sup>3,4</sup> In agreement, the c.6527insC mutation recurrence has also been recently disclosed in the RDEB population in Chile where waves of Spanish migration occurred at different historical periods.<sup>5</sup> Notably, this mutation has also been found in isolated patients from France<sup>6</sup> and Germany,<sup>7</sup> two countries with a long history of mid-20th century Spanish immigration.

Herein, the genetic history of this recurrent mutation was further investigated in a cluster of Spanish patients with RDEB carrying the c.6527insC mutation using three mathematical approaches. The age of the most recent common ancestor of the c.6527insC chromosome was estimated to be around 40 generations old, and the mutation first occurred about 120 generations ago. As a result, the first mutational event occurred 80 generations before the first common ancestor arose. Assuming a generation time of 28 years,<sup>8</sup> the time at which the c.6527insC mutation first occurred was between 3204 and 3416 years ago (13th–15th century BC). In this period, various pre-Roman communities settled in the southern half of the Iberian peninsula (the Spanish geographic area with a high c.6527insC recurrence). The estimated age of the most recent common ancestor is about 1131 years ago suggesting that the founder effect occurred between the late first to early second millennium AD. Notably, it coincides with a 'golden age' of Sephardic Jews in Al-Andalus, a closed inbred community.<sup>9</sup> Due to the fact that c.6527insC is a mutation that has not been identified among Sephardic Jewish populations, we hypothesize that

families carrying the c.6527insC mutation belonged to the group of Jews who converted to Catholicism and remained in Andalusia after being expelled from Spain in the 15th century (bottleneck population).

This study was performed by expanding the intragenic haplotype construction previously reported<sup>3</sup> on a large segment of 2.1232 cM (centimorgans) flanking the COL7A1 gene on chromosome 3 (3p21.31). Seven tag single nucleotide polymorphisms (SNPs) (highly associated SNPs) were selected (Fig. 1a) for the construction of extragenic haplotypes in a total of 25 index cases and 48 parent–offspring trios from the general Spanish population (see Data S1). By using this approach, a highly conserved extragenic haplotype (E08) was identified in 86.11% of the c.6527insC alleles from unrelated patients with RDEB (Fig. 1b) confirming the existence of a single common ancestral chromosome as previously suggested.<sup>3</sup> The c.6527insC chromosomes have also been linked to other extragenic haplotypes (E18, E06, E03 and E14), which, like E08, share the same intragenic haplotype (H5). Additionally, the identification in the general Spanish population of a total of 37 different extragenic haplotypes (E01–E37) reveals a high genetic variability associated with this region (Data S2). Discordant distribution of haplotypes between the control and the c.6527insC carrier groups suggests a very large genetic distance between them. In fact, we estimated the age of the most recent common ancestor of the Spanish chromosomes bearing this mutation is 40.40 generations (Fig. 2).

This age was estimated by employing the method developed by Colombo.<sup>10</sup> This method takes into account the association between every tag SNP and the mutation (linkage disequilibrium), the tag SNP's frequency in disease and normal chromosomes, genetic distances between tag SNPs and the mutation (cM), the c.6527insC carrier frequency (as a part of the proportion of population sampled,  $f$ -value) as well as the population growth rates ( $r$ ) (Data S3). As predicted, the European global demographic growth rate ( $r = 0.05$ ) was not equivalent to the growth rate of Spain ( $r = 0.076$ ). Taking into account these differences, and assuming the same proportion of population was sampled ( $f = 0.000412$ ), the allelic age is 47.23 generations, with a difference of seven generations compared with the age estimated using the Spanish growth rate. Moreover, due to the c.6527insC mutation being prevalent among patients from the southern half of the Iberian peninsula<sup>3</sup> ( $f = 0.000847$ ), the age calculated with the population growth rate in this given area ( $r = 0.0676$ ), was 40.39 generations. The fact that the resulting values of the most

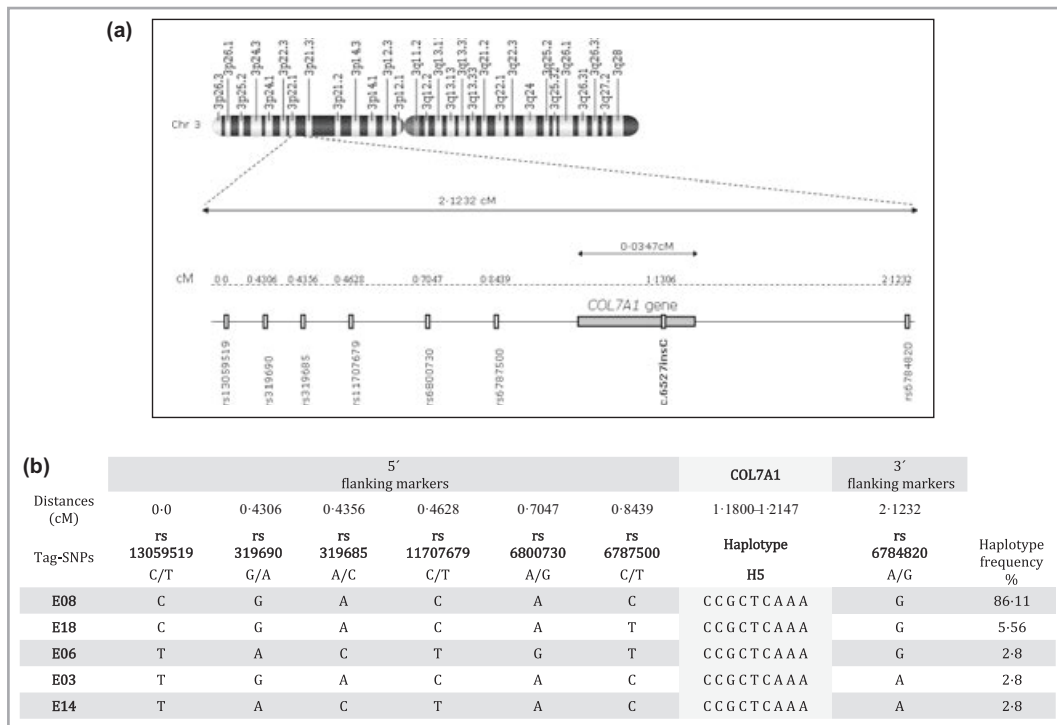


Fig 1. Haplotype analysis. (a) Schematic representation of the genetic distances between seven selected single nucleotide polymorphisms (SNPs) flanking the COL7A1 locus in a 2.1232 cM region. (b) Extragenic COL7A1 haplotypes associated with c.6527insC constructed by manual separation of alleles after SNP genotyping (patients and their parents). All carriers of the mutation shared the same intragenic H5 ancestral haplotype (CCGCTCAA\_6527insC).

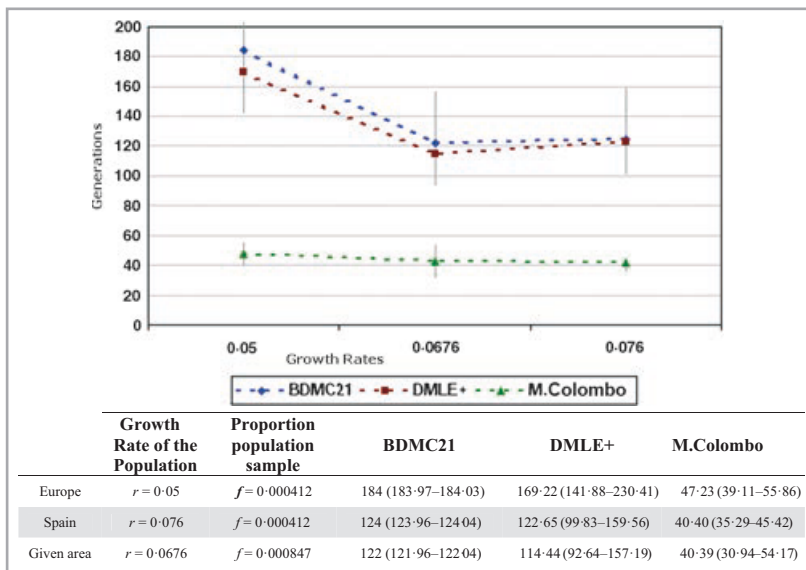


Fig 2. Graphic illustration of the estimated age by using BDMC21, DMLE+ and the method described by Colombo<sup>10</sup> (see text). Note that the first two methods determine the time at which the mutation first occurred while the third method calculates the age of the most recent common ancestor. The growth rate of the population is a critical point in the DMLE+ and BDMC21 methods, but has little effect on the Colombo method.

recent common ancestor carrying the c.6527insC mutation when considering the growth rates of Europe, Spain and the given area, were not drastically different gives an idea of the consistency of our calculations (Fig. 2).

We also estimated the time at which the c.6527insC mutation first occurred by using two different mathematical software programs, DMLE+ (<http://www.dmle.org>) and

BMCD21 (<http://www.rannala.org>) (Fig. 2). Both programs take into consideration map distances between SNPs and mutation site (cM; Fig. 1a), the c.6527 carrier frequency (included in  $f$  calculations) and population growth rates ( $r$ ) (Table S3B in Data S3). DMLE+ software also considers haplotype data from affected patients and unaffected controls, unlike BDMC21. The DMLE+ and BMCD21 methods revealed

that the c.6527insC mutation first occurred 122.65 and 124 generations ago, respectively. The estimated allelic age fluctuated with the  $r$  value, rendering differences that ranged between 47 and 62 generations as shown in Figure 2. These results show that the population growth rate is a critical parameter for DMLE+ and BDMC21 estimates unlike the minor effect of this rate in the Colombo calculations.

In conclusion, by expanding the haplotype construction on a large segment of 2.1232 cM flanking the COL7A1 gene, we were able to confirm unequivocally that the c.6527insC mutation originated from a single ancestor. Moreover, this mutation arose for the first time around 3300 years ago and it penetrated into a bottleneck population about 1131 years ago. These estimates coincide with the historical outline of the Spanish population. To our knowledge this is the first study dating a mutation in the COL7A1 gene. In our opinion, carrier screening for the c.6527insC COL7A1 mutation should be encouraged in couples with ancestry from the Iberian peninsula in analogy with Tay–Sachs disease in persons of European–Jewish ancestry (recommendations of the American College of Obstetricians and Gynecologists; <http://www.acog.org>).

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## Supporting information

**Data S1.** Single nucleotide polymorphism selection and haplotype construction. Table S1 Primers and conditions for amplification of different genetic markers. List of restriction enzymes to characterize single nucleotide polymorphisms.

**Data S2.** Table S2 Extragenic COL7A1 haplotypes in the general Spanish population.

**Data S3.** Population growth rates ( $r$ ) and proportion population sample ( $f$ ) in different populations. Linkage disequilibrium (LD) analysis between c.6527insC and markers. Table S3A LD analyses for the c.6527insC mutation and markers flanking the COL7A1 locus. Table S3B Estimation of the time of the most recent common ancestor of the c.6527insC chromosomes in two different areas (given area and Spain).