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METHODS LETTER TO THE EDITOR

WILEY Experimental Dermatology

# Genome-wide single nucleotide polymorphism-based autozygosity mapping facilitates identification of mutations in consanguineous families with epidermolysis bullosa

## Abstract

Autozygosity mapping (AM) is a technique utilised for mapping homozygous autosomal recessive (AR) traits and facilitation of genetic diagnosis. We investigated the utility of AM for the molecular diagnosis of heterogeneous AR disorders, using epidermolysis bullosa (EB) as a paradigm. We applied this technique to a cohort of 46 distinct EB families using both short tandem repeat (STR) and genome-wide single nucleotide polymorphism (SNP) array-based AM to guide targeted Sanger sequencing of EB candidate genes. Initially, 39 of the 46 cases were diagnosed with homozygous mutations using this method. Independently, 26 cases, including the seven initially unresolved cases, were analysed with an EB-targeted next-generation sequencing (NGS) panel. NGS identified mutations in five additional cases, initially undiagnosed due to the presence of compound heterozygosity, deep intronic mutations or runs of homozygosity below the set threshold of 2 Mb, for a total yield of 44 of 46 cases (95.7%) diagnosed genetically.

## 1 | BACKGROUND

The diagnosis of heritable disorders, particularly genodermatoses, is complicated by genetic, phenotypic and clinical heterogeneity. Routine genetic diagnosis was originally based on PCR amplification and sequencing of exons and flanking introns in candidate genes identified by prescreening tools, in addition to clinical presentation. More recently, next-generation sequencing (NGS) methods, such as targeted panels and whole-exome/genome sequencing, have been incorporated into the diagnosis of these disorders.

A number of screening methods, taking advantage of the extensive polymorphism of the human genome, have been shown to facilitate the identification of candidate genes.[1] Autozygosity mapping (AM), initially based on restriction fragment length polymorphisms or short tandem repeat (STR) microsatellite markers, has been used to map recessive traits in consanguineous families.[2,3] While STRs are highly polymorphic, with up to 30 distinct alleles for each STR,[4] they are not amenable for high-throughput analysis. The advent of high-density single nucleotide polymorphism (SNP) arrays has allowed genome-wide mapping of regions of homozygosity (ROH) at high resolution for a relatively low cost in consanguineous and outbred (eg in the case of a founder effect) families.[1,5,6] AM can be used alone for mapping of causative genes before mutation analysis by Sanger sequencing or in combination with NGS to improve the mutation detection rate.

Epidermolysis bullosa (EB), the paradigm of skin fragility disorders, is caused by mutations in as many as 20 genes, most of which cause disease in a biallelic manner.[7-9] While EB can be classified into the subtypes of EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler syndrome (KS) based on the layer of cutaneous blistering, there is significant phenotypic variability within this group of disorders,[7] especially in early life. Given the heterogeneity of EB, it serves as a model disease to assess the functionality of AM in facilitating genetic diagnosis. We applied STR and whole-genome SNP-based AM in 46 families (Families 1-46) with EB with a consanguineous background from Iran, a country in which 39% of marriages are consanguineous.[10] To provide a full investigation of the merits of AM in our cohort of EB patients, this study reports on a combination of previously unreported patients in our cohort and previous results.[11-13]

## 2 | QUESTIONS ADDRESSED

We investigated the clinical utility of AM for the molecular diagnosis of Mendelian disorders with extensive genetic heterogeneity in a cohort affected by EB with a high degree of consanguinity.

## 3 | EXPERIMENTAL DESIGN

See Data S1 for complete materials and methods.

## 4 | RESULTS

Following STR and SNP-based AM, alignment of EB-related genes with ROH in combination with clinical correlation identified putative candidate gene(s) in 43 of 46 families (Table 1, Table S1). Subsequent sequencing of the suggested gene(s) identified causative homozygous

**Abbreviations:** AM, autozygosity mapping; AR, autosomal recessive; DEB, dystrophic EB; EB, epidermolysis bullosa; EBS, EB simplex; JEB, junctional EB; KS, Kindler syndrome; NGS, next-generation sequencing; RDEB, recessive DEB; ROH, run of homozygosity; SNP, single nucleotide polymorphism; STR, short tandem repeat.

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**TABLE 1** Summary of genetic findings in EB cohort using autozygosity mapping (AM) followed by sequencing of candidate gene(s)

| | |
|---|---|
| Total cases | 46 |
| Candidate gene(s) identified by AM | 43 |
| No candidate gene identified | 3 |
| Homozygous mutation identified | 39 |
| EB simplex—AR | 5 |
| Junctional EB | 18 |
| Recessive dystrophic EB | 15 |
| Kindler syndrome | 1 |
| Novel variants | 8 |
| No initial mutation identified | 7 |
| Compound heterozygous | 2 |
| Mutation in <2 Mb block | 2 |
| Not targeted by STR mapping | 1 |
| Unknown | 2 |

AM, autozygosity mapping; EB, epidermolysis bullosa; AR, autosomal recessive; STR, short tandem repeat.

mutations in 39 families (84.7%). In the remaining families, sequencing of exon 1 in *KLHL24* failed to identify mutations in this recently described candidate gene.[8] The pathogenicity of missense mutations revealed in this study was determined by bioinformatics prediction programmes (Table S2).

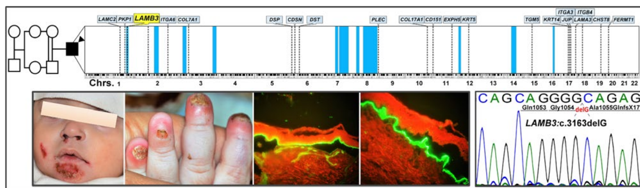

**FIGURE 1** Autozygosity mapping, representative clinical features, immunoepitope mapping and mutation analysis of the candidate gene in a case of laryngo-onycho-cutaneous syndrome. A SNP panel of 550 000 markers was used to identify homozygosity blocks of ≥2 Mb (vertical blue lines) along the entire autosome; chromosomes 1-22 are listed at the bottom. The genomic loci of candidate genes known to be associated with EB are indicated by vertical dotted lines as shown in the panel. Pedigree of a first cousin consanguineous marriage with autosomal recessive inheritance pattern and homozygosity mapping in the proband of Family 7, who was initially diagnosed with laryngo-onycho-cutaneous (LOC; Shabbir) syndrome. Note the cutaneous erosions and excess granulation tissue on the chin and nasal cavity as well as dystrophic changes in the nails. Only one EB-related gene co-aligned with a homozygosity block, implicating *LAMB3* on chromosome 1q32.2 (yellow box). Staining with a monoclonal antibody against integrin ß4 (MAB 1964, Millipore Co., Billerica, MA, USA) marked the blister roof (left) and with a monoclonal antibody against collagen VII (MAB 1345, Millipore Co.) marked the blister floor (right); localisation of these two proteins in immunofluorescence analysis indicated that the level of cleavage is within lamina lucida suggesting the diagnosis of JEB. Sanger sequencing analysis revealed the mutation c.3163delG (p.Ala1055Glnfs*17) in the *LAMB3* gene

As an example, Family 7 was referred with an initial diagnosis of laryngo-onycho-cutaneous (LOC, Shabbir) syndrome, usually caused by mutations in *LAMA3A*.[14] However, sequencing of *LAMA3* in another laboratory was unyielding. Sanger sequencing guided by SNP-based AM revealed a novel homozygous mutation in *LAMB3*:c.3163delG (Figure 1). Additional examples of this successful diagnostic approach are shown in Figures S1 and S2.

Seven unresolved cases of 46 remained. In three of these families (Families 4, 11 and 35), no ROH for EB-associated genes was identified. In four other families (Families 21, 23, 32 and 42), initial sequencing failed to identify a causative variant despite the identification of candidate genes using ROH. There are several factors contributing to the lack of initial identification of mutations, which call attention to potential pitfalls of AM.

First, the parameters utilised to overlap ROH with candidate genes can affect the detection rate. For example, in Families 11 and 35, no candidate genes were initially identified in ROH ≥2 Mb. However, eventual NGS identified a homozygous deletion in *COL7A1* and a homozygous nonsense mutation in *LAMB3*, respectively. Further analysis revealed that these mutations reside within ROH of less than 2 Mb.

Second, there were two cases (Families 4 and 32) in which AM with subsequent sequencing failed to identify a causative mutation due to the presence of a compound heterozygous mutation, raising the issue that, while extremely rare in highly consanguineous populations, compound heterozygosity can lead to misleading findings.

The lack of genetic diagnosis in the three remaining cases was likely due to a combination of factors. In Family 23, two affected siblings with nephrotic syndrome, interstitial lung disease and skin fragility were found to have a single overlapping ROH of 12 Mb harbouring *ITGA3*. While this gene has been associated with this phenotype of kidney, lung and skin disease,[15] subsequent NGS failed to identify mutations within exons or the exon-intron junctions. This suggests that probably there is a deep intronic mutation affecting *ITGA3* expression in this family, and RNA sequencing can further delineate the molecular pathology in this case. In Family 42, NGS identified a causative homozygous nonsense mutation in *PLEC* (p.Arg2424*). *PLEC* was not present in our STR library and thus was not identified as a candidate gene in this case. In Family 21, a case of lethal neonatal EB, while *COL17A1* was suggested by AM, subsequent NGS was unyielding.

Of note, the yield of 84.7% using AM-guided sequencing was remarkably similar to that of the independent use of our previously reported NGS-targeted panel,[12] in which potential causative variants were identified in 76 of 91 (83.5%) families with EB, the AM approach being expedient and cost-efficient in case of consanguinity in the families. Additionally, combining AM with targeted NGS panels has significantly improved our mutation detection rate. Use of AM can narrow down the list of potential variants from an NGS panel and can provide additional evidence for the causative role of variants of unknown significance, such as missense and intronic variants. Finally, it should be noted that NGS data can be used to generate SNP-based homozygosity maps.

## 5 | CONCLUSIONS

The use of AM in Mendelian disorders with extensive genetic heterogeneity in consanguineous populations is a highly effective method for mutation detection, comparable to a multigene NGS panel for EB. We propose that AM can have a similar efficacy for other heterogeneous disorders with autosomal recessive inheritance, in isolation or in combination with NGS techniques.


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### CONFLICT OF INTEREST

The authors have declared no conflict of interest.

### AUTHOR CONTRIBUTIONS

LY and HV initiated the study, collected samples and performed the mutation analysis; AT, AS, JB, HH, CK, MH and SZ assisted in mutation analysis; JU, AT and HV wrote the manuscript; JU oversaw the project; MD, MA, SN, NA, SS, NM and HM assisted in clinical evaluation and genetic counselling.


**Keywords**
autosomal recessive Mendelian disorders, epidermolysis bullosa, genodermatoses, homozygosity mapping, autozygosity mapping



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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**Data S1.** Supplementary Material
**Table S1.** Autozygosity mapping and genotyping of Iranian families affected by epidermolysis bullosa
**Table S2.** The pathogenicity of missense mutations revealed in this study as determined by bioinformatics prediction programs
**Figure S1.** Autozygosity mapping, representative clinical features, immunoepitope mapping and mutation analysis of candidate genes in junctional EB
**Figure S2.** Autozygosity mapping, representative clinical features, immunoepitope mapping and mutation analysis of candidate genes in EB simplex patients