

Genetic variants in human *BCL2L11* (*BIM*) are associated with ulcerative forms of Buruli ulcer

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ABSTRACT



Buruli ulcer (BU) is a devastating skin mycobacterial infection characterized by extensive cell death, which was previously suggested to be mediated by Bcl2-like protein 11 (BIM, encoded by the *BCL2L11* gene). We here report the association of genetic variants in *BCL2L11* with ulcerative forms of the disease in a cohort of 618 Beninese individuals. Our results show that regulation of apoptosis in humans contributes to BU lesions associated with worse prognosis, prompting for further investigation on the implementation of novel methods for earlier identification of at-risk patients.

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Buruli ulcer (BU) is a skin disease typically prevalent in tropical countries, with over 20000 cases diagnosed in the past decade worldwide [1]. Although its global incidence has been decreasing, local foci of the disease in countries such as Ghana or Australia have been displaying an increasing number of BU notifications, leading to public health concern [1]. The disease is caused by *Mycobacterium ulcerans*, which produces mycolactone, a macrolide-like toxin that is able to induce cellular apoptosis [2]. Upon infection, patients develop a small skin lesion – nodule, ulcer, plaque or oedema – that slowly and painlessly may progress to larger disfiguring ulcerative lesions as a consequence of extensive cellular apoptosis and necrosis [2,3]. The transition to these more severe forms of the disease was shown to depend on variables other than time, likely related to the host–pathogen genetic interplay [3]. In this regard, mice with absent expression of pro-apoptotic mediator *Bcl2l11* revealed enhanced resistance to *M. ulcerans*-induced tissue ulceration [2]. Thus, we sought to determine whether genetic variants in human *BCL2L11* could associate with the risk of developing BU or the progression to ulcerative forms of the disease in a Beninese cohort.

We performed this study in accordance with the ethical standards of the Helsinki Declaration. The National Ethical Review Board of the Ministry of Health in Benin approved the collection and analysis of blood samples [clearance Nu 018, 20/Oct/2011; registration number IRB0006860]. We selected SNPs (Supplemental Table 1) based on their ability to tag surrounding variants in the HapMap-YRI population of the International HapMap project, phase III, NCBI (National Center for Biotechnology Information) build 36 [4]. To this aim, we used Haploview version 4.2 software [5] to assess linkage disequilibrium blocks with a pairwise correlation coefficient $r^2 \geq 0.8$ and a minor allele frequency (MAF) > 0.05 , using the confidence intervals method and the LD-plot function. Considering a power estimation of 80% [6] – significance level of 0.01, additive disease model, disease prevalence of 0.02, MAF of 0.07, and genotype relative risk of 2.0 –, we expanded the cohort previously described in [7] with new cases diagnosed up until 2017, to a total of 618 age- and gender-matched cases (309) and controls (309). A final minimum call rate $> 97\%$ was obtained for all the SNPs genotyped. Using PLINK version 1.07, we assessed Hardy-Weinberg equilibrium with the χ^2 test and

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Table 1. Genotype distributions and association test results of SNPs in the BCL2L11 gene among BU patients and healthy endemic controls (left) and among BU patients with non-ulcerative and ulcerative forms of the disease (right).

| SNP rs# | Genotype | Controls (%) | | OR [95% CI] | t-statistic | p-value | Empirical p-value | Non-ulcerated (%) | | Ulcerated (%) | OR [95% CI] | t-statistic | p-value | Empirical p-value | Adjusted empirical p-value |
|-------------------|----------|--------------|------------|---------------------|-------------|---------|-------------------|-------------------|---------------|---------------------|-------------|-------------|---------|-------------------|----------------------------|
| | | Cases (%) | (%) | | | | | Non-ulcerated (%) | Ulcerated (%) | | | | | | |
| rs1821968 | GG | 133 (42.9) | 131 (42.4) | 0.913 [0.719-1.162] | -0.735 | 0.462 | 0.502 | 29 (36.7) | 95 (44.6) | 1.264 [0.851-1.878] | 1.159 | 0.246 | 0.264 | 0.656 | |
| | GA | 145 (46.9) | 146 (47.7) | | | | | 41 (51.9) | 98 (46.0) | | | | | | |
| | AA | 32 (10.4) | 32 (10.5) | | | | | 9 (11.4) | 20 (9.4) | | | | | | |
| rs17041869 | AA | 260 (84.1) | 257 (83.2) | 1.040 [0.707-1.529] | 0.197 | 0.844 | 0.795 | 63 (79.7) | 183 (85.9) | 1.461 [0.796-2.684] | 1.223 | 0.221 | 0.206 | 0.596 | |
| | AG | 45 (14.6) | 49 (16.0) | | | | | 15 (19.0) | 28 (13.1) | | | | | | |
| | GG | 4 (1.3) | 3 (1.0) | | | | | 1 (1.3) | 2 (0.9) | | | | | | |
| rs9308731 | AA | 233 (75.4) | 235 (76.1) | 0.898 [0.650-1.239] | -0.656 | 0.512 | 0.508 | 64 (81.0) | 156 (73.2) | 0.697 [0.405-1.199] | -1.305 | 0.192 | 0.182 | 0.530 | |
| | AG | 66 (21.4) | 70 (22.9) | | | | | 13 (16.5) | 49 (23.0) | | | | | | |
| | GG | 10 (3.2) | 4 (1.3) | | | | | 2 (2.5) | 8 (3.8) | | | | | | |
| rs13421194 | TT | 250 (80.9) | 241 (78.0) | 1.122 [0.786-1.603] | 0.635 | 0.526 | 0.481 | 57 (72.2) | 181 (85.0) | 2.017 [1.157-3.518] | 2.474 | 0.013 | 0.016 | 0.048 | |
| | TC | 54 (17.5) | 65 (21.2) | | | | | 20 (25.3) | 30 (14.1) | | | | | | |
| | CC | 5 (1.6) | 3 (1.0) | | | | | 2 (2.5) | 2 (0.9) | | | | | | |

performed genetic associations by means of logistic regression, under an additive model of inheritance and taking as reference the major allele [8]. Empirical *p*-values were further calculated through 5000 Monte Carlo permutations, provided the random seed “1473879600”, and adjusted for multiple comparisons. We explored SNP features using the Ensembl browser, release 100 [9]. Transcription factor binding sites (TFBS) were predicted with the PROMO software, using version 8.3 of TRANSFAC, and considering a maximum dissimilarity rate of 15 [10].

One SNP, rs1980045, displayed a significant deviation from the Hardy-Weinberg principle and was subsequently excluded from further analyses ($p < 0.0001$). Upon comparison of the genotype distribution across groups, according to the presence of BU history and the type of lesion manifested, we found the T allele at rs13421194 (OR = 2.017; 95% CI = 1.157-3.518; $p = 0.013$) to be associated with a two-fold increase in the odds of developing ulcers (Table 1). Intronic SNPs are increasingly being recognized as important modulators of genetic expression [11]. In silico analysis predicted rs13421194 to possess binding sites to Signal transducer and activator of transcription 4 (STAT4) and to c-Ets-1 (Supplemental Figure 1), which have been implicated in several cellular processes, including autophagy and mycobacterial growth inhibition [12], survival of lymphoid cells, regulation of cytokine and chemokine-related pathways, and apoptosis during the angiogenesis process [13]. We have also found that rs13421194 overlaps *MIR4435-2-HG* (MIR4435-2 host gene, ENSG00000172965.17) (Supplemental Figure 2), a long non-coding RNA (lncRNA) whose homologous in mice, *Morrid*, regulates the transcription of *Bcl2l11*, particularly in short-lived myeloid cells, both in physiological and infectious contexts [14]. On the other hand, lncRNAs have been found to strongly associate with the development of BU in a recent genome-wide association study [15]. These findings thus highlight novel putative mechanisms of regulation of *BCL2L11* expression during *M. ulcerans* infection, commendable of exploration in future studies.

Our study was based on a cohort of relevant sample size and adequately matched taking into consideration known confounding variables. Results here obtained are in agreement with the above-mentioned GWAS, in that rs13421194 was not significantly involved in BU acquisition (OR = 1.193; 95% CI = 0.901-1.579; $p = 0.214$, major allele as reference), although it remains to be known if data on the association with the ulcerative phenotype can also be replicated. Cell death mediators modulate BU pathophysiology and it is now more evident that such process is governed by a complex network of *BCL2L11*-associated SNPs. Because of the low cost and speed of DNA genotyping, the new knowledge here presented may be regarded in

the stratification of the risk of ulceration among BU patients. This in turn enables more informed decisions on optimal individual follow-up intervals and on the timing to escalate treatment strategies, potentially modifying the course of the disease.

Disclosure statement

No potential conflict of interest was reported by the author.

Data availability statement

The data that support the findings of this study are available from the corresponding author, A.G.F., upon reasonable request.

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