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 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 *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 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.
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

| SNP ref | Genotype Cases (%) | Controls (%) | Genotype OR [95% CI] | t-statistic | p-value | Empirical p-value | Adjusted empirical p-value |
|---------|-------------------|-------------|----------------------|------------|---------|------------------|----------------------------|
| rs1821968 | GG | 133 (42.9) | 131 (42.4) | 0.913 [0.719-1.162] | -0.735 | 0.462 | 0.502 |
| rs17041869 | AA | 260 (84.1) | 257 (83.2) | 1.040 [0.707-1.529] | 0.197 | 0.844 | 0.462 |
| rs9308731 | AA | 233 (75.4) | 235 (76.1) | 0.898 [0.650-1.239] | -0.656 | 0.512 | 0.635 |
| rs13421194 | TT | 250 (80.9) | 241 (78.0) | 1.122 [0.786-1.603] | 0.635 | 0.526 | 0.536 |
|          | TC | 54 (17.2)  | 56 (17.3)  | 1.223 [0.925-1.629] | 0.304 | 0.764 | 0.767 |
|          | CC | 10 (3.2)   | 12 (3.9)   | 1.095 [0.707-1.748] | -1.424 | 0.154 | 0.154 |

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).
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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