Are *BIC* (miR-155) Polymorphisms Associated with Eczema Susceptibility?

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Eczema (nomenclature according to the World Allergy Organization (1), also referred to as atopic dermatitis, OMIM#603165) is one of the most common chronic inflammatory skin disorders. Twin studies indicate a strong genetic contribution to the development of eczema, and genetic linkage analyses have identified several chromosomal regions and candidate genes linked to eczema susceptibility (2).

The discovery of microRNAs (miRNAs) has raised the question whether not only classical genes but also non-coding RNA genes may be involved in the pathogenesis of eczema. miRNAs are short (20–22 nt), non-coding RNAs, which suppress the activity of their target genes at the post-transcriptional level (3). miRNAs have important regulatory roles in virtually all biological functions, including development, cell proliferation, immune response and tumourigenesis. miRNA research has been a subject of interest in the dermatology field recently, and intriguing results have demonstrated the importance of miRNAs in skin biology (4–8). We have shown that skin from patients with eczema and psoriasis has an altered expression pattern of miRNAs compared with healthy skin (4, 9). However, the question still remains whether genes coding for miRNAs may be genetically associated with eczema susceptibility.

In searching for genes that are differentially expressed in eczema and healthy skin, we recently performed a large-scale gene-expression study (10). As shown in Fig. 1a, the *BIC* (B-cell Integration Cluster) transcript was found to be among the consistently over-expressed transcripts in eczema skin compared with healthy control skin. Furthermore, we demonstrated that *BIC* expression was induced by the application of *Malassezia sympodialis* to non-lesional skin of eczema patients (Fig. 1b; for experimental details see (10)). The *BIC* gene lacks any long open reading frame, which implies that it functions as an untranslated RNA. Interestingly, it encodes a non-coding RNA with an evolutionarily conserved secondary structure, which serves as the precursor of miRNA-155 (miR-155). This miRNA plays crucial roles in innate and adaptive immunity, inflammation and cancer (11). The increased level of *BIC* transcript in eczema skin is in agreement with our recent results obtained from the miRNA expression profiling of atopic eczema skin, showing that miR-155, the only known miRNA to be derived from the *BIC* transcript, is overexpressed in eczema skin (9).

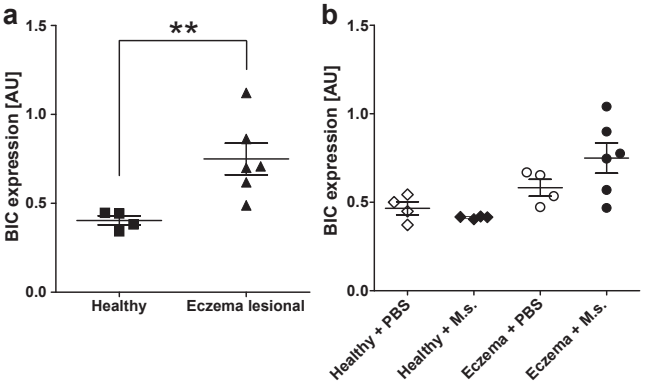

Fig. 1. BIC/miRNA-155 transcript is increased in eczema skin. The B-cell Integration Cluster (BIC) transcript is among the over-expressed genes that were identified by cDNA microarrays when analysing the global gene-expression profiles in eczema and healthy skin (10) by a multi-group significance analysis of microarrays approach. From the full microarray dataset, we have here extracted and described the BIC transcriptional profile in detail. (a) BIC mRNA levels in healthy ($n=4$) and lesional eczema ($n=6$) skin samples. $^{**}p<0.01$; Mann–Whitney test. (b) BIC transcript levels were measured after 48 h application of *M. sympodialis* yeast extract (M.s.) on both non-lesional eczema and healthy control skin. Phosphate-buffered saline (PBS) was used as negative control. Individual values are plotted and the standard error is shown. (Healthy + PBS, $n=4$; non-lesional eczema + PBS, $n=5$; non-lesional eczema + *M. sympodialis*, $n=6$ and Healthy + *M. sympodialis*, $n=4$). AU: arbitrary units.

Since the *BIC* transcript maps to an asthma and eczema susceptibility region on chromosome 21q21 (12, 13), it is intriguing to speculate that *BIC/miR-155* may be genetically associated with eczema susceptibility. To test this hypothesis, we genotyped 5 single-nucleotide polymorphisms (SNPs), tagging the *BIC/miR-155* chromosomal region (Fig. S1; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1466), using genotypes from HapMap release #22 mapped to NCBI build 36 (dbSNP126), which was available in November 2007, in a Swedish eczema cohort consisting of 406 multiplex eczema families, including a total of 1,514 individuals. The eczema material was recruited at the Karolinska University Hospital in Stockholm, during 1995 to 1997 (14). A subset of these patients (n=701; 46.3%) had eczema together with elevated allergen-specific IgE antibodies (age 6–20 years: > 263 kU/l; age > 20 years: > 122 kU/l) (14), i.e. atopic eczema (1).

As shown in Table I, there was a trend of association of the *BIC* gene with atopic eczema (rs987195, $p<0.04$; rs1893650, $p<0.02$; rs928883, $p<0.02$), however, this

Table I. *Five single nucleotide polymorphisms (SNPs) covering the B-cell Integration Cluster (BIC) locus on chromosome 21 were tested for genetic association to eczema susceptibility. The* BIC *gene was found to be associated with atopic eczema, i.e. in a subset of eczema patients having raised levels of allergen-specific IgE antibodies. However,* p-*values were not statistically significant after correction for multiple testing (Bonferroni-correction;* p-*values shown are not permutated)*

SNP	Position	MAF	Minor allele	*p*-value	OR (95%CI)
rs969885	25853004	0.04	A	0.092	0.72 (0.49–1.06)
rs987195	25859193	0.04	G	0.035	0.58 (0.34–0.99)
rs1893650	25862686	0.21	T	0.018	0.74 (0.58–0.96)
rs928883	25865896	0.11	A	0.019	0.62 (0.41–0.95)
rs1547355	25869610	0.05	A	0.314	0.71 (0.35–1.40)

Genotype data for the BIC gene region (21q21.3) was downloaded from the HapMap project (http://www.hapmap.org) and analysed in the Haploview program (www.broadinstitute.org/haploview). SNPs were selected using the Tagger feature in Haploview using default settings. Genotyping and allelic discrimination was performed as previously described (15). The Unphased (3.0.10) program was used for pedigree disequilibrium test (PDT) analysis and odds ratio (OR) estimates including 95% confidence interval (CI) (http://homepages.lshtm.ac.uk/frankdudbridge/software/unphased).
MAF: minor allele frequency.

proved not to be significant upon correction for multiple testing (Bonferroni correction). One of these SNPs (rs1893650), which is located in the putative promoter region of the *BIC/miRNA-155* gene, also showed a significant *p*-value in the whole eczema data-set ($p = 0.05$), including atopic and non-atopic eczema patients (data not shown). There was no association with rs1547355, which is not located in the promoter region or the gene itself, but in downstream genomic sequence. In summary, none of the nominally significant *p*-values were significant upon multiple testing.

Results from experiments performed with miR-155 knockout mice have demonstrated that it has essential roles in B- and T-cell responses and the organization of dendritic cell – T-cell interactions. Interestingly, miR-155-null mice had fewer class-switched antibodies after immunization compared with control mice and showed reduced IL-2 and IFN-γ production indicating that deletion of miR-155 compromised multiple aspects of adaptive immunity. We have identified miR-155 to be overexpressed in atopic eczema, mainly by activated CD4+ T cells, and shown that it is involved in the regulation of T-cell activation and proliferation via regulating 1 of its targets, cytotoxic T-lymphocyte antigen-4 (CTLA-4) (9). In summary, we show here that the *BIC* gene, the only known precursor of miR-155, is more highly expressed in lesional skin and in *M. sympodialis* patch-tested non-lesional skin of eczema patients compared with skin of healthy controls. Data presented here support our earlier results, but do not prove a genetic association between the *BIC/miR-155* gene and atopic eczema. However, SNPs in the *BIC/miR-155* gene may affect the expression/processing of miR-155 and contribute to susceptibility to eczema. Further research is needed to determine whether genetic variations in miRNA genes, or in their targets, may contribute to the susceptibility to chronic inflammatory diseases.

The authors declare no conflicts of interest.

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