Psoriasis is associated with increased β-defensin genomic copy number

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Psoriasis is a common inflammatory skin disease with a strong genetic component. We analyzed the genomic copy number polymorphism of the β-defensin region on human chromosome 8 in 179 Dutch individuals with psoriasis and 272 controls and in 319 German individuals with psoriasis and 305 controls. Comparisons in both cohorts showed a significant association between higher genomic copy number for β-defensin genes and risk of psoriasis.

In humans, β-defensins are small, secreted, antimicrobial peptides, which are encoded by *DEFB* genes in three main gene clusters: two on chromosome 20 and one on 8p23.1 (ref. 1). Of the eight β-defensin genes at 8p23.1, *DEFB1* (encoding the protein hBD-1) and *DEFB103* (encoding the protein hBD-3) are expressed constitutively in skin, and *DEFB4* (encoding the protein hBD-2) can be induced in cultured keratinocytes by cytokines or bacterial lipopolysaccharides[2]. The β-defensin genes on 8p23.1, with the exception of *DEFB1* but including *DEFB4*, *SPAG11*, *DEFB103*, *DEFB104*, *DEFB105*, *DEFB106* and *DEFB107*, are on a large repeat unit (**Supplementary Fig. 1** online) that is variable in copy number[3]. Individuals have between 2 and 12 copies per diploid genome, with a modal copy number of four in the UK. The antimicrobial and proinflammatory nature of these β-defensins suggests that quantitative variation in gene dosage might contribute to susceptibility to infectious and inflammatory disease[4].

Psoriasis is a common inflammatory skin disease with a prevalence of about 2% in the populations of developed countries. It has both environmental and genetic components to its etiology, and linkage analysis has been used to identify multiple loci and alleles that confer risk of the disease, with the strongest genetic effect found at 6p21.3, where haplotypes carrying the HLA-*Cw6* allele are associated with an increase in risk[5]. Psoriasis is characterized by red-scaling, elevated plaques, commonly on the elbows, knees and trunk. Histological examination of psoriatic lesions shows inflammation and disturbed epidermal differentiation. hBD-2 is induced as a part of the inflammatory response in skin, which, in psoriasis, is part of the regenerative maturation process involving hyperproliferation and induction of marker genes such as those encoding elafin and cytokeratins 6, 16 and 17. In addition to their antimicrobial activity, it has been shown that hBD-2 and other skin β-defensins have cytokine-like properties[6]. The central role of these proteins in the innate immune system of the skin suggested that β-defensin genes could be candidate genes for psoriasis susceptibility.

Copy number variation at the 8p23.1 β-defensin cluster, commonly over the 2–7 copy range, poses greater challenges for accurate genotyping than variation at lower copy numbers. To investigate the relationship between β-defensin gene copy number and susceptibility to psoriasis, we initially used a multiplex amplifiable probe hybridization assay (MAPH; **Supplementary Table 1** online) to determine copy number of the β-defensin repeat per diploid genome[3,7,8]. Comparison of (unrounded) MAPH data from 190 Dutch affected individuals (cases) and 303 controls suggested an association between increased defensin gene copy number and psoriasis ($P = 1.65 \times 10^{-6}$, *t*-test). As an alternative assay for β-defensin gene copy number, we used the higher-throughput paralog ratio test (PRT) on the same set of samples; PRT typing of *DEFB4* copy number uses specific coamplification of a heat-shock protein pseudogene upstream of *DEFB4* together with a single-copy paralog on chromosome 5 and has been described previously[9]. Comparison of PRT results from 179 Dutch cases and 272 controls also suggested an association with psoriasis, but at a lower significance ($P = 0.01$, *t*-test).

Although capable of high throughput, a single PRT assay has an error rate[9] for *DEFB4* copy number estimated at about 8%. True copy number values are likely to be integers. We therefore sought to improve the overall accuracy of copy number determination in this cohort by combining information into a consensus integer copy number for 179 cases and 272 controls, using data from MAPH, PRT and ratios of multisite variants (MSVs)[7,10] mapping around the *DEFB4* gene (dbSNP reference numbers rs2740091, rs2737532 and rs3762040) (restriction enzyme digest variant ratios, REDVR; see **Supplementary Methods** online). Integer copy number agreed between MAPH/REDVR and first-pass PRT for 78% of samples and for a further 11% on repeat PRT typing of discordant samples. This consensus integer copy number was significantly higher among cases than among controls (**Fig. 1a**; $P = 7.8 \times 10^{-5}$, *t*-test).

As an independent test of this association, we typed 305 controls and 319 cases from Germany using PRT alone. Analysis of PRT results showed a significant association with both unrounded ($P = 9.02 \times 10^{-6}$, *t*-test) and integer-rounded analyses (**Fig. 1b**; $P = 2.95 \times 10^{-5}$,
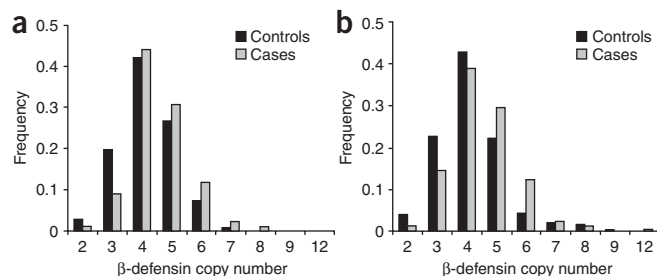
Figure 1 Frequency distributions of β-defensin genomic copy number. (**a**) Dutch controls and psoriasis cases. (**b**) German controls and psoriasis cases.

t-test). We do not believe that our findings can be attributed to differential genotyping bias[11] because, in addition to the independent replication, important details of genotyping, including sample preparation, missing data and integer clustering, were closely matched between cases and controls for both cohorts (see **Supplementary Methods**, sections C1 and C2). We also carried out detailed comparisons between independent typing platforms for the Dutch samples, and we believe that our copy number typing attained a high standard of accuracy (see **Supplementary Methods**, section C3). The full set of data for all samples typed is available as **Supplementary Table 2** online. There was no significant difference between the Dutch and German cohorts for controls ($P = 0.165$, *t*-test) or cases ($P = 0.95$, *t*-test). Assuming a population prevalence of 2%, the relative risk of psoriasis for each copy number class can be inferred, with 1.00 representing the mean population risk (**Table 1**). Combining both cohorts, there is significant support ($P = 0.005$, linear regression analysis of variance (ANOVA), weighted by the square root of sample size) for a specifically linear model in which each additional copy above two copies increases the relative risk, and the linear regression equation suggests that each copy adds 34 percentage points (95% confidence interval: 25–43%) to the relative risk.

We cannot exclude the possibility that it is the nucleotide state of an MSV that actually causes susceptibility to psoriasis, and that copy number is only indirectly associated, as a proxy for this sequence variant. For the variants we have studied, in an extended Dutch cohort, psoriasis shows no association with rs3762040 ($P = 0.958$) and only weak association ($P = 0.043$) with rs2740091. However, we note that more than 200 substitutional

variants are reported in dbSNP for this region and were not tested here.

Because of the cytokine-like properties of β-defensins, either high resting levels or high induced levels may be a precipitating factor after minor skin injury, infection or some other environmental trigger. This could lead to an inappropriate inflammatory response, generating the clinical symptoms typical of psoriasis. It has been shown that the abundance of hBD-2 in keratinocytes after induction by cytokines is correlated with its basal expression level[12], and hBD-2, hBD-3 and hBD-4 have all been found to stimulate keratinocytes to release IL-8, IL-18 and IL-20, proinflammatory cytokines that have an established role in the etiology of psoriasis[13]. The genes encoding hBD-2, hBD-3 and hBD-4 (*DEFB4*, *DEFB103* and *DEFB104*, respectively) are all part of the β-defensin repeat region and show the same copy number, so we cannot distinguish whether one gene or a combination of all three genes is responsible for the gene-dosage association with psoriasis.

We have identified a psoriasis susceptibility locus not previously identified by linkage analysis. We therefore considered whether the strength of the effect observed in our data should give rise to a detectable linkage signal. We simulated pedigrees with the same β-defensin haplotype population frequencies and subject to the same dependence of risk on β-defensin gene copy number genotype (**Table 1** and **Supplementary Methods**). Only a minority of simulated studies involving 500 affected sibling pairs detect linkage at even 'suggestive' levels of significance ($P < 0.05$). Similarly, simulation of sibling recurrence risk (λ_s) suggested that the β-defensin locus accounts for a locus-specific λ_s of about 1.08. This low value largely reflects the common occurrence of the variation, and the fact that within pedigrees, total diploid copy number may not have a simple relationship with inheritance of parental alleles. At this level of effect, loci are undetectable by even large linkage studies and may only be discovered by candidate-gene association studies.

Copy number polymorphism of the cytokine gene *CCL3L1* is reflected in expression levels and has been shown to influence susceptibility to HIV-1 infection[14], and low copy number of complement C4 genes was associated with systemic lupus erythematosus in a recently published study[15]. We adopted PRT as a high-throughput method to genotype loci such as these, with copy numbers of 2–12, to determine discrete copy numbers while maintaining a level of accuracy comparable to that of MAPH. Given the number of loci that vary in copy number across the genome,

Table 1 Influence of β-defensin gene copy number on the relative risk of psoriasis

Copy number	Dutch controls	Dutch cases	German controls	German cases	Combined controls	Combined cases	Copy number	Population frequency (%)	Relative risk[a]	95% CI relative risk
2	8	2	12	4	20	6	2	3.5	**0.31**	0.12–0.77
3	54	16	69	46	123	62	3	21.3	**0.51**	0.37–0.71
4	115	79	131	124	246	203	4	42.6	0.84	0.67–1.05
5	73	55	68	94	141	149	5	24.4	1.08	0.83–1.40
6	20	21	13	39	33	60	6 or more	8.1	**1.69**	1.16–2.48
7	2	4	6	7	8	11				
8	0	2	5	4	5	6				
9	0	0	1	0	1	0				
12	0	0	0	1	0	1				
Sum	272	179	305	319	577	498				

Counts are given in the table, together with the relative risk of psoriasis given the observed copy number, with a relative risk of 1.00 representing the population mean risk (2%). Population frequency shows an estimate of diploid copy number frequencies in native Northern Europeans deduced from the 'combined controls' column.
[a]Relative risk values were calculated after pooling copy numbers of 6 or more into a single category. Bold type indicates a two-tailed significant difference from a relative risk of 1.00 at the 5% level.

and the large number of these loci that are candidates for susceptibility to different diseases, large-scale copy number typing of case-control cohorts will be a priority.

Note: Supplementary information is available on the Nature Genetics website.

AUTHOR CONTRIBUTIONS

E.J.H., A.R., J.S. and J.A.L.A. conceived and coordinated the study. P.C.M.v.d.K., H.T., M.d.H., P.L.J.M.Z., D.R.-O., A.R. and J.S. collected and characterized the clinical samples. Copy number typing was carried out by E.J.H. (MAPH/REDVR), R.P., J.A.L.A., J.L. and U.H. (PRT). E.J.H., J.S., J.A.L.A. and G.d.J. performed the statistical and typing quality analysis, with contributions from P.L.J.M.Z., U.H. and M.d.H. All authors contributed to the writing of the paper, with major input from E.J.H., J.S., A.R. and J.A.L.A.

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