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Cytogenetic analysis of 477 psoriatics revealed an increased frequency of aberrations involving chromosome region 11q

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Psoriasis is an inflammatory skin disorder affecting approximately 3% of the population. Genetic studies published so far have shown a complex genetic inheritance with heterogeneity and a putative major susceptibility locus in the HLA region on chromosome 6. We have collected a large amount of material consisting mostly of small nuclear families in order to perform a genome-wide scan for psoriasis-associated genes. In order to focus the scan properly on possible candidate regions, we performed a cytogenetic analysis of 477 unrelated psoriatics. We divided our findings into sporadic, affecting a minor fraction of the cells, and constitutional, ie they were present in all cells examined. We found three cases of balanced translocation, all of which involved chromosome 11q. Two of these had a breakpoint in q12–13, whilst one involved the telomeric part of chromosome 11q. In order to characterise further the breakpoint on 11q12–13, we used bacterial artificial chromosomes (BACs) analysed by fluorescent *in situ* hybridisation (FISH). We were able to show that the persons had a close, but not identical breakpoints; they were separated by at least 5 cM. The major atopy locus is located in this region, as well as a locus for insulin-dependent diabetes mellitus, both being conditions with a pathogenetic mechanism involving antigen presentation.

Keywords: psoriasis; cytogenetics; translocation; FISH

Introduction

Psoriasis is a common skin disorder with a complex genetic background. We have previously published a large population study indicating a recessive mode of inheritance in a Swedish patient sample.¹ The genetic heterogeneity of this condition is well recognised and, as in several other immunologically mediated diseases,

there is evidence for a gene within the HLA region.² The genetic linkage analyses presented so far also indicate several possible non-HLA gene locations, all of which have not been confirmed.^{2–6} Two of these studies using a few large pedigrees presented data suggesting a psoriasis susceptibility locus on 17q and 4q respectively.^{3,4} Trembath *et al*⁵ recently reported a genome scan of 66 affected sib pairs in which four potential psoriasis susceptibility loci were identified, on chromosome 2p, 6p (HLA region), 8q and 20p. Nair *et al*⁶ confirmed the locus in the HLA region and presented two additional novel loci on chromosome 16q and 20p using 182 sib pairs in a genome-wide scan.

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We have recently confirmed linkage to 6p and 17q, but not to 4q in the Swedish patient sample.⁶ However, a number of factors make the interpretation of these studies difficult. These include the complex inheritance pattern, differences between population groups, incomplete penetrance, the existence of phenocopies, and the possibility of misdiagnosis. By using a candidate gene approach, ie concentrating attention on genes potentially involved in the pathogenesis of psoriasis due to their function, these problems can be circumvented to some extent.

Another method of identifying candidate gene regions is to search for chromosomal abnormalities pointing towards a genetic defect involved in the pathogenesis of the disease. Cytogenetic studies have in this way contributed to the identification of several disease genes. Two well-known examples are the Duchenne muscular dystrophie (*DMD*) gene in Xp21⁷ and the neurofibromatosis type 1 locus in 17p,⁸ where translocations and deletions helped in identifying and cloning the gene. To our knowledge, this approach has so far not been successfully used in genetically complex diseases.

We now report the results from the cytogenetic analysis of 477 unrelated individuals with psoriasis. The main purpose of this study was to focus a genetic linkage analysis of a large psoriasis population on a possible candidate region. We also wanted to estimate the quantity and nature of chromosomal abnormalities present in a psoriasis population. We report on the increased frequency of chromosomal aberrations in the psoriasis patient sample, in particular the involvement of chromosome region 11q in three constitutionally balanced translocations.

Materials and Methods

Material

From a total of 22 000 questionnaires sent out to the members of the Swedish Psoriasis Association, data were collected from 14 008 families. Blood samples were collected from 104 families where at least two siblings were affected. Both parents were unaffected in accordance with a recessive model. The material is described in detail elsewhere.^{1,9} To check the diagnosis of the proband, all individuals were examined by one dermatologist (AI) using well established diagnostic criteria. They were also asked about disease history, other diseases and medication. The median age among the probands was 41 years. The 104 families to which they belonged are being used in a current linkage analysis. Cytogenetic analysis was performed in one affected sibling from each family. Blood samples from another unrelated 373 persons with psoriasis were also included in the study.

These patients are used in disequilibrium studies. These persons are also members of the Swedish Psoriasis Association. Cytogenetic analyses were also performed for the parents of three psoriatics with a translocation involving chromosome 11q.

Cytogenetic Analysis

Cytogenetic analyses were performed in a total of 477 unrelated psoriatics. Spreads from metaphase chromosomes were prepared from cultured peripheral blood lymphocytes according to a modified method described by Johanneson *et al*¹⁰ and analysed using G-banding technique with a resolution approximately at the 400 band level or greater. Between 5 and 30 mitoses were analysed for each sample (DH).

Isolation of Bacterial Artificial Chromosomes (BACs)

The Human DNA pool library, purchased from Research Genetics Inc., Huntsville, AL, USA, was screened with PCR-assays using primers for the high affinity IgE receptor gene, obtained from the GDB database, and for the polymorphic markers D11S913 and D11S1314, obtained from the Génethon map. According to the genetic map,¹¹ these markers are located approximately 5 and 10 cM distal to the IgE receptor gene on 11q, respectively. The BAC clones were cultured using standard procedures and extracted using the QIAGEN Plasmid Purification kit, Hilden, Germany.

FISH

The BAC DNA was labelled with Cy3-dCTP and nicktranslated according to the FluoroLink™ Cy3™ Nick Translation Kit (Amersham LIFE SCIENCE, Pittsburgh, PA, USA) and was hybridised to metaphase chromosome spreads of cultivated blood lymphocytes according to the procedures of the laboratory. The fluorescence *in situ* hybridisation was detected with a Zeiss Axiophot microscope and a computer-driven IMAC-CCD colour camera.

Results

Cytogenetic analyses were performed on one psoriatic sibling in each of 104 families and also on the other 373 unrelated psoriatic persons, in all 185 males and 292 females. The cytogenetic aberrations, divided into constitutional and sporadic, are listed in Table 1 and 2.

Table 1 Constitutional chromosomal aberrations

7 cases of inv(9)(p11q13)
3 cases of 1qh ⁺
2 cases of 22ph ⁺
15cenh ⁺
1 case of t(4;11)(q21.3;q25) and 15cenh ⁺
t(7;11)(q11.23;q13.5)
t(1;11)(p36.3;q12.2)
1 case of +21
13ph ⁻
9qh ⁺

Table 2 Sporadic chromosomal aberrations

1 case of t(1;12), t(13;15) del1p, 45,X,-Y
t(15;19)
t(5;13)(q13;q34)
t(1;15)(q11;q11)
t(X;13)(p11;q11)
1 case of t(7;14)(p11;q11)
t(7;14)(p11;q11)
t(7;14)(q32;q11.2)
t(7;14)(q34;q11)
t(7;14)(q32;q13)
1 case of del11q13
del 1q11
del 10q23
del 9q12
del 5p15
del 14q22
del 9p21
del 10q22
1 case of +4
+13
1 case of inv1p
2 cases of 15ph ⁺
3 cases fra(X)(p22)
1 case of fra(2)(q37)
fra(9)(q31.1)
48XX,del(17)(p11),+2mar
47XX,+mar
16 cases of 45,X
6 cases of 45X/46XX/47XXX
1 case of 47XXX
49XXXXXX
47XXY

The constitutional abnormalities affect all the 30 analysed cells. In contrast, the sporadic aberrations only affect a minor fraction of the cells, commonly only one (mosaic). Many of the findings can be regarded as normal variations without known pathological significance. In three psoriatics a translocation involving chromosome 11 was found. One had the breakpoint in the telomeric part of the chromosome t(4;11)(q21.3;q25). The other two had almost identical breakpoints in 11q12–13, ie t(7;11)(q11.23;q13.5) and t(1;11)(p36.3;q12.2). These three persons were young, unrelated Swedish psoriatics living in the southern part of Sweden. They all had an early age of onset ($m = 17$ years) and the diagnosis was confirmed by a dermatologist (AI). They had no history of other immunological diseases and did not take any medication at the time of the cytogenetic analysis. Two of them had one unaffected sibling and the third had no siblings. Because all three of them had unaffected parents and

no affected siblings, the segregation of the translocation with the disease could not be followed in these families. For these psoriatics, blood samples were available from the parents. We found that the t(7;11) was a *de novo* translocation, the t(1;11) was present in the unaffected mother, whereas the t(4;11) was present in the unaffected father.

In order to characterise these translocations further, fluorescent *in situ* hybridisation was performed with three bacterial artificial chromosomes (BACs) containing the β -subunit of the IgE receptor gene (a), genethon marker D11S913 (b) and D11S1314 (c), respectively. The distance between the markers was approximately 5 cM. FISH analysis using these BACs as probes showed that for the individual with t(1;11), the breakpoint was located between the BACs a and b, and for the individual with t(7;11) it was located distal of probe c (Figure 1E). Among the sporadic findings (Table 2), we draw attention to an individual with a deletion of 11q13, thus with the same breakpoint as in the two mentioned above, but only in one mitosis. Each of five persons with psoriasis had a translocation (7;14) in one cell; all of them had breakpoints in chromosome 14, (14q11–13). These five individuals did not take any medication, such as methotrexate, nor did they have any other diseases that could explain the finding.

Discussion

Psoriasis is a skin disorder with an association to the HLA region. The psoriatic skin lesion is characterised by hyperproliferation of the epidermis together with an inflammatory cell infiltration. Owing to the association with certain HLA antigens, the first attempts to find linkage in population studies were made in this region. By now, several reports confirming the likely presence of a major susceptibility locus in the HLA region have appeared.^{2,5,6} This places psoriasis in the same category as certain other conditions with a strong immunomediated pathogenetic component such as celiac disease, insulin-dependent diabetes mellitus and atopy.

The Breakpoint in 11q25

Celiac disease is an HLA-associated disease with immunomediated intolerance to gluten resulting in a malabsorption in the small intestine. There is a strong association with the HLA region but, once again, only part of the familial aggregation is explained by the HLA component.¹² A recently published genome-wide scan indicated the presence of two non-HLA loci, 5pter

and 11qter.¹³ The marker used for the latter, D11S934, is mapped to the chromosomal region 11q23–24 according to the GDB (Amplimer) database. Based on these reports, we suggest that the finding of a translocation

breakpoint on 11q25 in a psoriatic person could be non-random. Trembath *et al*⁶ also showed suggestive linkage in this region for psoriasis with the maximal *P* value for the marker D11S910 in the telomeric part of the

Figure 1 FISH analyses of translocations in psoriatics **A** *t*(1;11) A BAC containing the IgE receptor gene in 11q is hybridised to a metaphase spread and analysed with FISH. The probe is located on 11q and *t*(11p)(1q), thus proximal to the breakpoint. **B** *t*(1;11) A BAC containing the marker D11S913 located 5 cM distal to the BAC used in A is used as a probe in the same metaphase. The probe is located on 11q and *t*(1p)(11q), thus distal to the breakpoint. **C** *t*(7;11) A BAC containing the IgE receptor gene is hybridised to a metaphase spread and analysed with FISH. The probe is located on 11q and *t*(11p)(7q); thus proximal to the breakpoint. **D** *t*(7;11) A BAC containing marker D11S913 is used as a probe; it is still located proximal to the breakpoint, ie in 11q and *t*(11p)(7q). **E** Location of BACs in relation to chromosomal breakpoints in 11q in two psoriatics.

chromosome, ie 11q25. There are some indications of a higher prevalence of celiac disease among psoriatics,¹⁴ which increases the significance of our finding. However, our own data based on linkage analysis of 134 sib pairs do not show any statistically significant *P* values on chromosome 11q (manuscript in preparation).

The Breakpoint in 11q12–13

Insulin-dependent diabetes mellitus is another example of an autoimmune disease with a major susceptibility locus in the HLA region. It is of interest that one of the non-HLA loci, IDDM4, has been located to chromosome 11q13.¹⁵

There is also a genetic basis for atopy. As early as in 1988, Cookson *et al* reported a locus on chromosome 11q12 using linkage analysis.¹⁶ There have also been reports of linkage of one of the high affinity IgE receptor genes, located in this region, to clinical atopy,¹⁷ although several groups later failed to confirm this locus in other populations.¹⁸ It is regarded as the major atopy candidate gene in this region and it is responsible for the allergic response through the release of inflammatory mediators. We here report two cases of a translocational breakpoint in this subchromosomal region. We used a BAC clone isolated using this gene in a FISH analysis and showed that the gene is located proximal to the breakpoint in both individuals. Using two BACs containing markers located further downstream, we showed that the breakpoints are closely located, but not identical. At least 5 cM separates the two breakpoints.

Translocations between Chromosome 7 and 14q11–13

Five individuals had a translocation (7;14) in one cell out of 30 with the same breakpoint, 14q11–13, where the alpha and delta subunit of the T-cell antigen receptor are located. The significance of the findings, however, is not clear, since there have been reports of sporadic rearrangements involving chromosome 7 and 14 in normal lymphocyte culture.¹⁹ The T-cells are 'MHC-restricted' in that they identify foreign antigen only in combination with the proper HLA antigen. A dysfunctional T-cell receptor could, in conjunction with a certain HLA antigen (Cw6), contribute to the inappropriate inflammatory response in psoriasis. We therefore suggest that the significance of our finding cannot be excluded.

Breakpoints in 1p36.3, 4q21.3 and 7q11.23

A two stage genome-scan indicated the presence of a gene locus in 7q11.23 for inflammatory bowel disease.²⁰ According to a recent publication on non-HLA loci in autoinflammatory diseases, there are no reports of such loci in 1p36.3 and 4q21.3.²¹

There are early reports of chromosomal aberrations in small psoriasis patient samples. A significantly higher frequency of gaps, breaks, dicentric and acentric chromosome fragments was found in 10 psoriatics.²² There have also been reports of cytogenetic abnormalities in psoriatic patients treated with methotrexate.²³ A large patient sample is required to overcome the factors that may influence the frequency of these aberrations, such as treatment, infection and the chromosome preparation technique. We examined 5 to 30 cells in each of 477 persons with psoriasis and found several cases of constitutional aberrations that are regarded as normal variations.²⁴ A number of single aberrations occurring only in one cell in one person could be regarded as coincidental or culturing artefacts, because of the very large patient sample examined. Against this background of abnormalities, some findings emerge that could point towards a chromosome fragility leading to a translocation. The breakpoint could indicate the location of a disease-causing gene.

There are several hypotheses to explain why balanced translocations have abnormal phenotypes. Submicroscopic deletions in the breakpoints is one explanation and gene disruption resulting in a nonfunctional gene is another. It is also possible that the expression of the translocated gene is altered due to influence of genes in the translocated position. If the translocation is of causative significance, it implies that a susceptibility gene for psoriasis could be located at the breakpoints on chromosome 11q12–13.

The coincidence of psoriasis and two translocations with an almost identical breakpoint suggests that the translocation is possibly of causal significance for psoriasis in these individuals. There is a possibility that the same gene is affected in both patients. For example, the disruption of a specific gene in one of the patients and a position effect of a translocated segment on the gene in the other patient could lead to the same phenotype due to the inactivation of the same gene. The region 11q12–13 could be considered a region of clustering of non-HLA susceptibility candidate loci in human autoimmune diseases since there are indications

of an atopy locus, as well as an IDDM locus in this subchromosomal region.

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