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Assignment of the gene locus for severe congenital neutropenia to chromosome 1q22 in the original Kostmann family from Northern Sweden

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Abstract

Autosomal recessive severe congenital neutropenia (SCN) or Kostmann syndrome is characterised by reduced neutrophil counts and subsequent recurrent bacterial infections. The disease was originally described in a large consanguineous pedigree from Northern Sweden. A genome-wide autozygosity scan was initiated on samples from four individuals in the original pedigree using high density single nucleotide polymorphism (SNP) genotyping arrays in order to map the disease locus. Thirty candidate regions were identified and the ascertainment of samples from two additional patients confirmed a single haplotype with significant association to the disorder ($p < 0.01$) on chromosome 1q22. One affected individual from the original Kostmann pedigree was confirmed as a phenocopy. The minimal haplotype shared by affected individuals spans a candidate region of 1.2 Mb, containing several potential candidate genes.

Keywords

Kostmann syndrome; severe congenital neutropenia; SNP array; homozygosity mapping

Introduction

Kostmann syndrome, or severe congenital neutropenia (SCN, MIM 202700), constitutes a primary immunodeficiency syndrome associated with reduced neutrophil counts. Untreated patients develop recurrent life-threatening bacterial infections but treatment with granulocyte colony-stimulating factor (G-CSF) normalises neutrophil numbers and reduces the incidence

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of infections dramatically [1–4]. Autosomal recessive SCN was first described in a large consanguineous kindred from Northern Sweden in 1956 [5]. Kostmann reported on six children with severe neutropenia in peripheral blood and a maturation arrest at the promyelocyte/myelocyte stage in the bone marrow [5,6]. A few affected members from the pedigree have survived and show an accelerated apoptosis of myeloid bone marrow progenitor cells through a mechanism that remains to be elucidated [7].

SCN constitutes a heterogeneous group of disorders, where most cases with congenital neutropenia have a dominant mutation in the gene encoding neutrophil elastase (*ELA2*) [8,9]. Dominant SCN may in a few individuals be caused by mutations in the *GFI1* gene, which encodes a transcriptional repressor [10]. In contrast, no mutations have been identified in the *ELA2* or *GFI1* genes in autosomal recessive SCN [9–11]. Interestingly, one single affected individual from the original Kostmann family carries a heterozygous *ELA2* mutation [12]. It has been suggested that this individual represents a phenocopy for Kostmann disease since the *ELA2* mutation is absent in samples from the remaining patients in the family [12]. Homozygosity for a mutation in the *MAPBP1P* gene, encoding the endosomal adaptor protein p14, has recently been identified in a pedigree with autosomal recessive congenital neutropenia [13].

The relatively low density of microsatellite markers has hampered the use of autozygosity mapping for identification of disease genes in autosomal recessive disorders in founder populations. Single nucleotide polymorphisms (SNPs) are abundant polymorphic markers evenly distributed in the genome at an average distance of 1 per kb and have potentials in high-density high-throughput genotyping strategies for disease gene mapping [14]. It has been estimated that about two SNP markers provide the same information as one microsatellite marker [15,16]. Although each biallelic SNP is less informative than a single microsatellite marker in most cases, a greater number of SNPs in aggregate can lead to higher information content at any particular point in the genome. The development of high-density SNP microarrays for genotyping has improved the possibilities for autozygosity mapping of rare disorders with founder mutations. In the present study, we performed a genome-wide autozygosity analysis on samples from four affected individuals in the original Kostmann pedigree using 10K and 100K SNP arrays. A haplotype on chromosome 1q22 was identified with a significant association to the disease.

Materials and Methods

Subjects

Blood samples were collected from members of the original Kostmann family (Family 1) for DNA extraction (Fig. 1). Samples were obtained from four affected and 17 healthy individuals in Family 1. In addition, DNA was extracted from formalin fixed paraffin embedded autopsy specimens from one deceased patient (P1, Fig. 1). We also included samples from one family with SCN (Family 2, Fig. 1) not connected to the Kostmann pedigree but from the same geographical region. Altogether, DNA was extracted from 27 individuals of whom six are diagnosed with SCN. All affected individuals had a history of severe persistent neutropenia, monocytosis, a maturation block at the promyelocyte/myelocyte level in the bone marrow, and a similar pattern of clinical signs and symptoms of recurrent fevers and bacterial infections. One affected individual (P2) has a more severe phenotype and she is a carrier of a mutation in *ELA2*. The parents and siblings to neutropenic patients are healthy with no history of frequent infections. Informed consent was obtained from all participant individuals and the study was approved by the ethical committee at Uppsala University.

Genetic mapping

SNP genotyping was performed with samples from four affected individuals in Family 1 (P2, P4, P5 and P6, Fig. 1) using the GeneChip Mapping 10K array (Affymetrix) and the GeneChip Mapping 100K array (Affymetrix) according to the manufacturers protocol. Homozygosity mapping and sorting of genomic regions were performed with dedicated software written in Matlab 7.0 (MathWorks), using both the physical size of the region covered by homozygous SNPs as well as the number of adjacent homozygous SNPs as selection criteria. Three cut-off parameters were used; *i*) regions where the homozygous interval spanned more than 1 Mb, *ii*) regions with ≥ 20 homozygous SNPs in all four patients genotyped or *iii*) regions with ≥ 30 homozygous SNPs in three out of four individuals, where one affected was excluded from the analysis at a time. The exclusion procedure was performed systematically in order to reduce the risk of false negatives due to recombinations close to the mutation and the possibility of a phenocopy. Homozygosity analysis was also performed for the gene loci of *ELA2*, *GFII* and *MAPBPIP*. Selected regions were further investigated by genotyping all members of both families with microsatellite markers derived from known and novel polymorphic repeats. Eight microsatellite markers, D1S2715, CTTT16, D1S305, CTGT, CA20, D1S1595, GA19 and AC16, were used to construct a haplotype on chromosome 1q22. Marker positions are 150.397, 150.840, 151.095, 151.442, 152.227, 152.250, 153.840, 153.923 Mb, respectively (NCBI Build 35.1). Microsatellite markers were amplified by polymerase chain reaction (PCR) with incorporation of fluorescent labels, as previously described [17]. PCR primers and conditions used are available upon request. Associations between marker alleles or haplotypes and the disease phenotype were calculated with both Fisher's exact test and P_{excess} [18] as the methods can complement each other [19]. The aggregation of families in the Northern Sweden suggests a high local carrier frequency, with an estimated a carrier frequency of 1/25 [5]. Unaffected parental haplotypes were therefore used as controls.

Results

Analysis of the *ELA2*, *GFII* and *MAPBPIP* loci for homozygosity excluded the genes as candidates for the disease in Family 1. Analysis for homozygosity in the 10K SNP genotypes of individuals P2, P4, P5 and P6 yielded no candidate regions that could be confirmed with microsatellite marker analysis.

Subsequent analysis of the 100K array genotypes resulted in the identification of 30 candidate regions. Homozygosity for one single region, on chromosome 1q22, could be confirmed with microsatellite markers on six out of eight chromosomes. The candidate interval covers 1.8 Mb where three patient samples investigated (P4, P5 and P6) were homozygous for the same SNP alleles. This corresponds to 108, 30 and 106 adjacent homozygous SNPs for P4, P5 and P6, respectively.

Individual P2 shared no continuous homozygous SNP pattern within this interval. Homozygosity for the region was confirmed on DNA from P1, P4, P5, P6 as well as in P7, derived from the non-related pedigree, using eight microsatellite markers (three known and five novel polymorphisms). Linkage analysis including healthy family members confirmed segregation of specific marker alleles with the disease with the exception of the nuclear family of P2. A haplotype constructed from microsatellite marker alleles segregates with the phenotype in both Family 1 and 2, except for patient P2, which does not carry the haplotype (Fig. 1). This is consistent with the SNP results.

The analysis of Family 2 revealed that the chromosome 1q22 region segregates with the disease and that it is identical by descent with four affected members of Family 1. This indicates a common founder mutation for SCN in the two families with the exception of P2. Association of single marker alleles to the disease was analysed and the strongest association was obtained

for D1S305 (p-values of 0.002 and 0.0004 with and without P2, respectively). The microsatellite marker haplotype was also found significantly associated with the disease (p-value <0.01, Table 1) and a four-marker haplotype yielded a maximum P_{excess} of 1.0 when P2 was excluded (Table 1). Haplotype analysis restricted the candidate region to an interval of 1.23 Mb between markers D1S2715 (P5) and rs1218577 (P6), at positions 150.397 Mb and 151.624 Mb, respectively (NCBI Build 35.1). This region spans 37 known genes.

Discussion

In this report we have used high-density SNP arrays to map the locus for severe congenital autosomal recessive neutropenia (Kostmann syndrome) to a 1.2 Mb region on chromosome 1q22. The region contains 37 known genes of which several encode proteins with functions that can be associated to the myeloid lineage and hence could play a role in the pathogenesis of SCN.

Both 10K and 100K SNP arrays (Affymetrix) were employed in the initial genome scan and the resolution obtained by the 100K arrays allowed us to identify a region that was confirmed with microsatellite markers. In retrospective, three patients (P4, P5 and P6) in the initial screen were homozygous for four SNPs in the 1q22 region on the 10K SNP array. However, the 10K array analysis resulted in several hundreds of such regions and these arrays have consequently an insufficient marker density for our sample set.

The haplotype associated to the disease is present on eight out of ten disease chromosomes in family 1 and the association is also supported by the analysis of the affected individual in family 2 from the same geographical area. None of the controls carry the disease associated haplotype. The one affected member of family 1 (P2) with deviating haplotypes is the only family member that carries a heterozygous *ELA2* mutation [12]. Interestingly, the phenotype of P2 differs in severity from other affected members in the family and she suffers from severe periodontal disease with alveolar bone loss despite treatment with G-CSF [4,20]. This is consistent with genetic heterogeneity in the Kostmann family with patient P2 being affected as a result of the *ELA2* mutation, whereas the remaining patients are homozygous for another mutation positioned within the 1.2 Mb on chromosome 1q22.

The SNP arrays have proven a very useful tool for identifying disease genes associated to a variety of disorders [21–24]. The method is highly advantageous, since it offers rapid high-throughput genotyping demanding only small amounts of DNA and the SNP resolution results in a limited need for subsequent time-consuming fine mapping. The mean inter-marker distance in the Affymetrix 100K array set is only 23.6 kb but the SNPs are not uniformly distributed through the genome. Some chromosomal regions are underrepresented on the arrays, especially telomeric and centromeric areas. This problem was encountered when analysing SNPs spanning the *ELA2* gene in the 19p13 region. This area is only sparsely covered by SNPs on the 100K array set and we therefore included additional microsatellite markers in the analysis to ensure a sufficient coverage on chromosome 19p13. We had *a priori* information about *ELA2* as a candidate gene, but in many situations genome-wide scans are initiated when no such information is available. This said, SNP arrays have improved the marker resolution dramatically compared to genome-wide scans using microsatellite markers.

Here we report the mapping of a gene for autosomal recessive severe congenital neutropenia (Kostmann syndrome) to chromosome 1q22 using samples from members the original Kostmann pedigree. SNP array analysis and haplotype analysis allowed us to narrow down the disease gene region to 1.2 Mb. We demonstrate that a genome-wide 100K SNP array screen using samples from three distantly related individuals may be sufficient to select candidate regions. We also show that this gene mapping approach is feasible despite genetic heterogeneity

in a very small sample set. More SCN families, especially from the Northern part of Sweden, are required to further narrow down the gene region. Meanwhile, a few out of the 37 genes in the region will be selected for mutation screening on the basis of their expression pattern, known function and potential role in the pathogenesis of SCN. Identification of the gene mutation behind the autosomal recessive SCN would improve diagnosis and it will also allow the development of cellular and transgenic models of SCN. This may advance our understanding of disease mechanisms and the development of novel treatments.

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Table 1

Association of chromosome 1q22 haplotypes to SCN. Distribution of three haplotypes (complete and partial) for microsatellite markers D1S2715-CTTT16-D1S305-CTGT-CA20-D1S1595-GA19-AC16 in affected individuals compared to controls.

Haplotype	All affected (n=6)		Without P2 (n=5)	
	Fisher's	P _{excess}	Fisher's	P _{excess}
4-4-5-2-4-3-2-2	0.0069	0.5	0.0054	0.6
4-4-5-2-4-x-y-z	0.0002	0.75	0.00006	0.9
r-4-5-2-4-x-y-z	0.00003	0.83	0.000005	1.0