Localization of the gene for Cowden disease to chromosome 10q22–23

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Cowden disease (CD) (MIM 158350), or multiple hamartoma syndrome, is a rare autosomal dominant familial cancer syndrome with a high risk of breast cancer. Its clinical features include a wide array of abnormalities but the main characteristics are hamartomas of the skin, breast, thyroid, oral mucosa and intestinal epithelium. The pathognomonic hamartomatous features of CD include multiple smooth facial papules, acral keratosis and multiple oral papillomas[1,2]. The pathological hallmark of the facial papules are multiple trichilemmomas[3]. Expression of the disease is variable and penetrance of the dermatological lesions is assumed to be virtually complete by the age of twenty[4]. Central nervous system manifestations of CD were emphasized only recently and include megalencephaly, epilepsy and dysplastic gangliocytomas of the cerebellum (Lhermitte-Duclos disease, LDD)[5–7]. Early diagnosis is important since female patients with CD are at risk of developing breast cancer. Other lesions include benign and malignant disease of the thyroid, intestinal polyps and genitourinary abnormalities[4,8–10]. To localize the gene for CD, an autosomal genome scan was performed. A total of 12 families were examined, resulting in a maximum lod score of 8.92 at $\theta = 0.02$ with the marker *D10S573* located on chromosome 10q22–23.

Twelve families participated in this study, including 40 affected individuals. Since CD has clinical manifestations similar to other phakomatoses such as neurofibromatosis and tuberous sclerosis, the initial search for linkage was concentrated on chromosomal regions known to contain tumour suppressor genes. We were able to exclude all the candidate loci, including *BRCA1*, *BRCA2* and the multiple endocrine neoplasia type 2 locus, *RET*.

Subsequently, a genome scan was initiated in five unrelated Dutch families: N1, N2, N3, N4 & N5. Testing 300 markers resulted in the exclusion of 85% of the genome. An indication of linkage (lod score >1) was obtained at four different loci: *AMY2B* (chromosome 1), *D3S1286*, *D10S573* and *APOCII* (chromosome 19). At two of these loci, the lod score exceeded 2 (*D3S1286* and *D10S573*). Using additional markers and family 0014, three of these loci could be excluded. For the fourth region, located on the long arm of chromosome 10, a statistically significant maximum lod score of 6.67 at $\theta = 0.03$ was obtained with marker *D10S215*. A more detailed analysis of this region was performed with the inclusion of seven additional families. Eight markers, localized to 10q22–23 (ref. 11), showed significant evidence for linkage to CD (Table 1). The highest lod scores were obtained with two markers: *D10S573*, $Z_{max} = 8.92$ at $\theta = 0.02$ and *D10S215*, $Z_{max} = 8.19$ at $\theta = 0.02$.

Haplotypes were constructed to define the most likely position of the Cowden gene within this region. The critical recombinants occurred in individuals N4-II-10 and 0014-III-3, both of whom have features of CD (as defined in Methods). The most likely position of the Cowden gene is in a 5 cM region between *D10S215* (N4-II-10) and *D10S564* (0014-III-3) (Figs 1, 2 and 3). The pattern of inheritance in all other families is consistent with this localization.

In family N2, individual II-11 inherited the unaffected haplotype (Fig. 1). Based on our dermatological criteria, he initially was considered affected. However, his clinical symptoms include a borderline number of keratotic lesions on the forearm and a few non-characteristic facial papules that were not examined pathologically. No other features of CD could be detected that, in contrast, are quite obvious in his affected relatives. Furthermore, both his daughters III-9 and III-10, aged 32 and 28 respectively, are free of symptoms, although one of them received the reconstructed unaffected grandmaternal chromosome. If, however, individual N2-II-11 is considered affected, the maximum lod score is reduced to 7.14 at $\theta = 0.04$.

There is no indication for genetic heterogeneity among the 12 families who originated from four different countries. CD in all the families, including four with LDD, showed linkage to 10q22–23. Although the pathognomonic dermatological features of CD are almost invariant, there is large clinical variation among families. An obvious example would be the presence or absence of LDD in CD families. The eventual isolation of the gene would resolve some of these issues pertaining to genotype–phenotype relationships.

No tumour suppressor gene or oncogene has yet been identified in the Cowden critical region. The presence of tumour suppressor genes on chromosome 10 has been indicated by loss of heterozygosity (LOH)

Table 1 Two-point lod scores between the CD locus and CA repeats markers

	Lod score (chromosome 10q22–23)							
locus/θ	0.00	0.05	0.10	0.20	0.30	0.40	θ_{max}	Lod_{max}
D10S580	−18.33	0.01	0.87	1.11	0.73	0.23	0.17	1.15
D10S605	−5.99	1.80	2.09	1.83	1.14	0.37	0.11	2.11
D10S569	−8.34	0.62	1.15	1.18	0.75	0.23	0.15	1.26
D10S607	−1.34	2.78	2.58	1.85	1.00	0.24	0.04	2.79
D10S219	3.26	7.02	6.41	4.73	2.81	0.96	0.03	7.14
D10S201	−5.96	2.83	2.96	2.42	1.44	0.43	0.09	2.98
D10S573	5.19	8.63	7.77	5.61	3.24	1.03	0.02	8.92
D10S215	4.40	7.99	7.24	5.30	3.09	0.97	0.02	8.19
D10S564	2.34	5.12	4.75	3.56	2.11	0.67	0.04	5.15
D10S583	−4.62	3.59	3.59	2.83	1.72	0.59	0.07	3.65
D10S574	−0.09	4.60	4.37	3.36	2.01	0.63	0.05	4.60

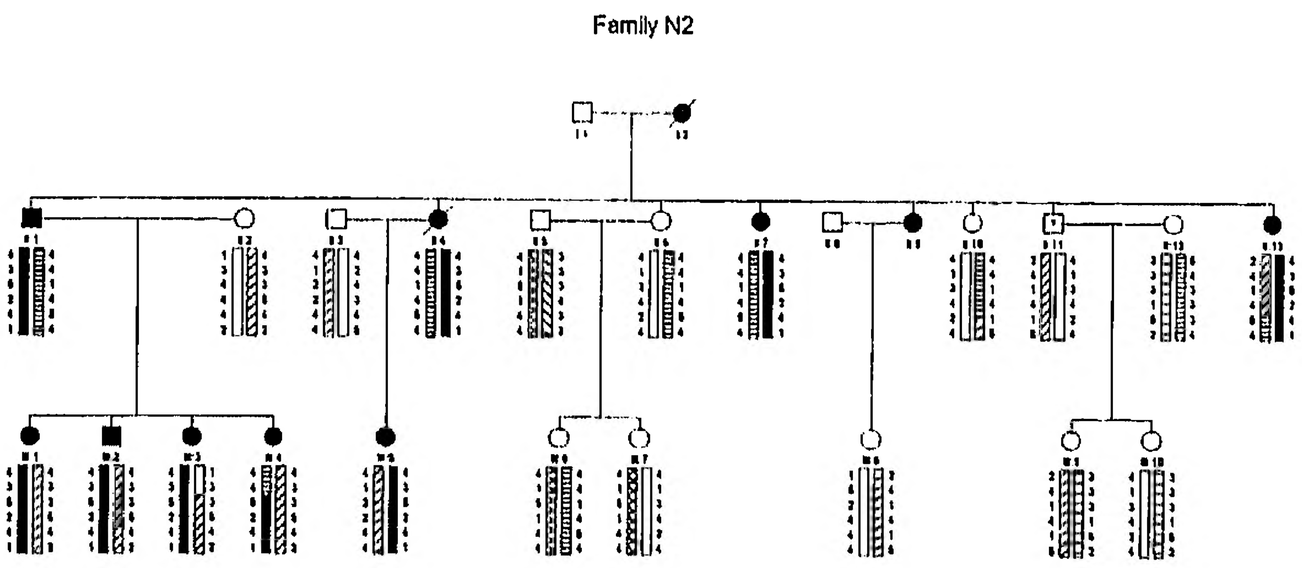

Fig. 1 Pedigrees of three Cowden disease families. Haplotypes of persons available for this study are shown. Bars indicate the chromosome and show regions of crossover. The mutation-carrying chromosome is depicted in black. Markers used to form this haplotype (proximal–distal): *D10S580, D10S219, D10S573, D10S215, D10S564* and *D10S583.*

in different types of tumours. Studies in follicular thyroid and uterine tumours, all components of CD, have shown frequent LOH on 10q (refs 12,13). The region of LOH was determined with more detail in endometrial tumours and the Cowden critical region may partially overlap this region[14].

There are several known genes mapped to the 10q22–23 region: *ACTA2*, *Glud1*, *INFI56* and *ZNF32* (GDB). Apart from *ZNF32*, the genes mentioned are not strong candidate genes. *ZNF32* is a member of the family *ZNF KOX* genes which encode the C_2H_2 Krüppel type (Class I) of zinc finger proteins[15]. There are at least two examples of inherited syndromes with developmental defects which are known to result from germline mutations in *ZNF* genes. The first is the *GLI3* gene in Greig cephalopolydactyly syndrome[16] and the second is the *WT1* tumour suppressor gene in Denys-Drash syndrome[17,18]. In addition, other ZNF-type proteins bind DNA at promoter sites and probably play important roles in gene regulation[19–23]. Fine mapping of *ZNF32* is necessary to determine if it maps within the Cowden critical region.

The two most severe complications for CD are neurological and neoplastic. There is insufficient information whether megalencephaly is caused by hypermyelination or by increased cerebral cellularity. Both hypermyelination and hypercellulairity are features consistent with the possibility that the Cowden gene might be a tumour suppressor gene. From an oncologic point of view, we suspect the *CD* gene might play a role in both familial and sporadic breast cancer and in familial thyroid syndromes. The high frequency of breast cancer in female Cowden patients (30%)[4,8–10] makes it a strong candidate gene for a new breast cancer susceptibility gene. In addition, its candidacy for the locus of non-medullary thyroid cancer should be considered. From this study, DNA-based predictive testing for CD in informative families is now possible, allowing early

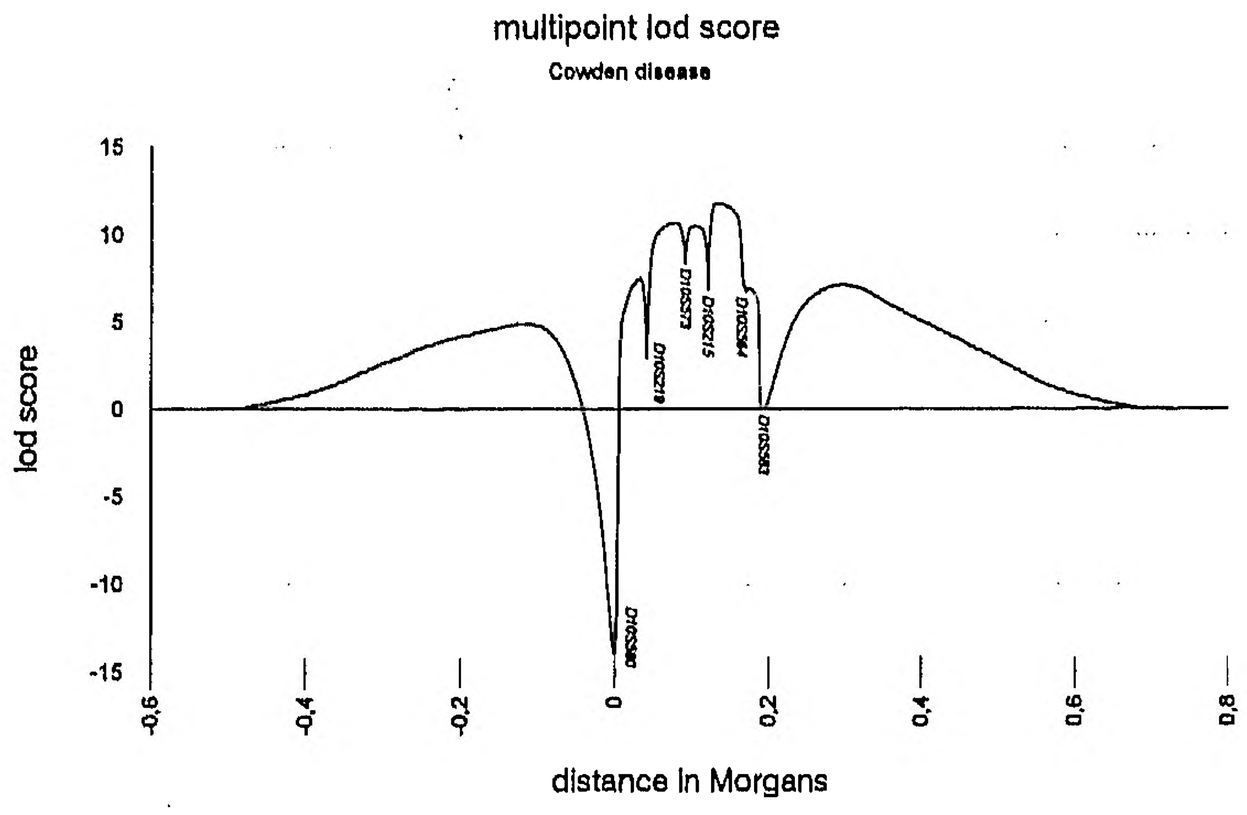

Fig. 2 Multipoint calculation. Markers used are flanking the Cowden critical region.

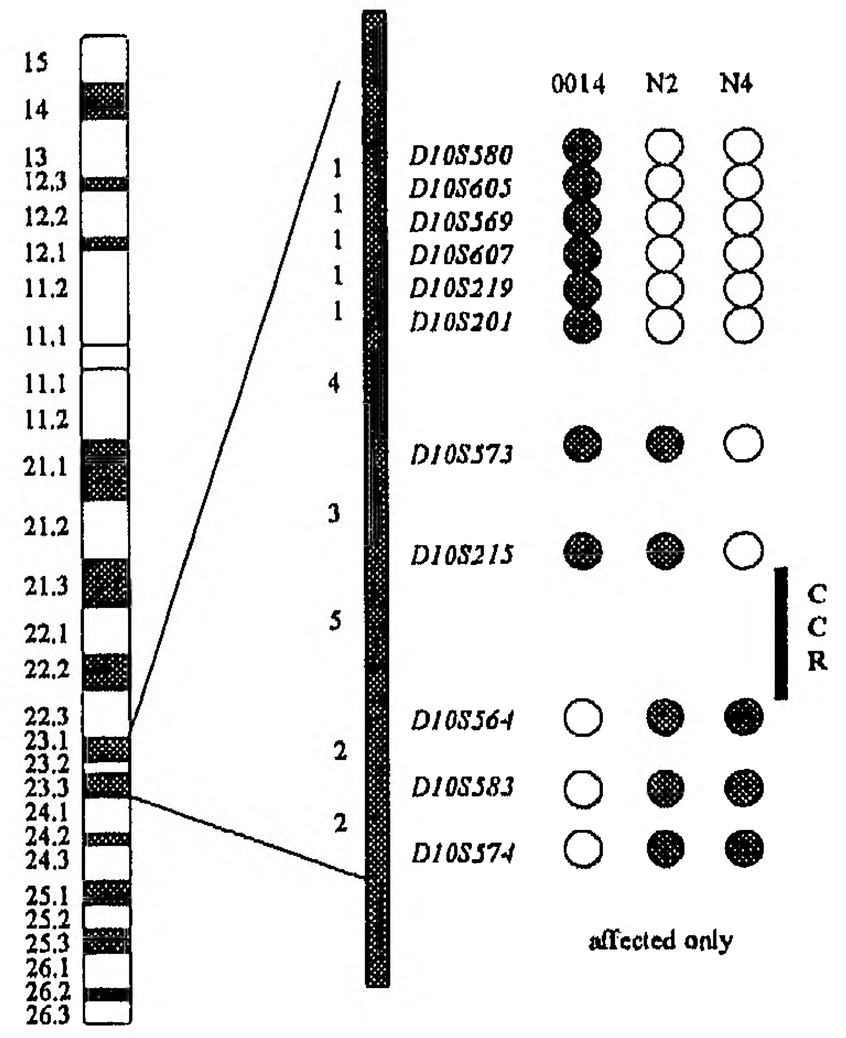

Fig. 3 Chromosomal localization of the Cowden critical region. Recombinant 0014-III-3, N2-III-4 and N4-II-10 are shown. Hatched circles indicate the affected haplotype, open circles indicate the unaffected haplotype. The Cowden Critical region is depicted as a black bar.

diagnosis and institution of possible early screening, such as for breast cancer.

Methods

Patients. Families N1, N2, N4, N5, 0014 and D have been described[4,5,7,24]. Family N3 was ascertained because the proband was diagnosed on clinical grounds to have LDD. The neurological and oncologic features of these families will be reported elsewhere in detail (Peeters *et al.* and Lin *et al.*, manuscripts in preparation). In addition, family D was ascertained because of LDD in the proband's grandfather[7]. For the linkage studies we used the operational criteria formulated by the international CD consortium.

International Cowden Consortium CD diagnosis criteria. Pathognomonic criteria for the diagnosis of Cowden disease include facial trichilemmomas, acral keratoses, papillomatous lesions and mucosal lesions. Breast cancer, thyroid cancer (especially PTC type), macrocephaly (97th percenile) and LDD were considered major criteria. Thyroid lesions (goitre), mental retardation (IQ ≤ 75), gastrointestinal hamartomas, fibrocystic disease of the breast, lipomas, fibromas and genitourinary tumours or malformations were applied as minor criteria. For the diagnosis of an individual the mucocutanous lesions are diagnostic if there are six or more papules, of which three or more must be trichillemmomas. Also cutaneous facial papules and oral mucosal papillomatosis, or oral mucosal papillomatosis and acral keratosis, or palmo plantar keratosis (≥ 6) are considered diagnostic for CD. Indicative criteria for CD include: two major criteria where one is either LDD or macrocephaly; one major with three minor criteria; or four minor criteria. All patients fulfill these criteria.

Typing of DNA markers. Genomic DNA used for the typing of the DNA polymophisms was isolated as described[25]. Amplification of the polymorphic regions and the separations of the amplified fragments was performed as described[26,27].

Statistical evaluation. Lod scores were calculated using the Linkage program (version 5.1)[28] subroutine MLINK for the two-point linkage. Multipoint calculations were done using FASTLINK (version 2.30), subroutine LINKMAP[29]. The gene frequency was estimated as 0.000001. Non-penetrance after the age of 20 was estimated to be 10%. Individuals above the age of 20 were typed and used for lod score calculation. For the individual N2-II-11, the affection status was said to be unknown.

Acknowledgements

We thank the family members who participated in our study; J. Schot, J. van Deutekom, A. Chompret, D. Lacombe, H. Sobol, J. Hurst and C. Toulouse for evaluating some of the families and for helpful discussions; C. Houghton, K. Healey and the CAOS/CAMM Centre for technical assistance. C.E. thanks J. Vijg for continued support and encouragement. M.R.N. is supported by a grant from the Faculty of Medical Science, the Department of Neurology, University Hospital Nijmegen; B.A.J.P. by a Gibb Fellowship of the Cancer Research Campaign (CRC); M.L. by Le Comité des Landes de la Ligue Nationale Francaise Contre le Cancer; and C.E. by the CRC Dana-Farber Cancer Institute Fellowship, the Lawrence and Susan Marx Investigatorship in Cancer Genetics in the Division of Cancer Epidemiology and Control, the Markey Charitable Trust, the Charles A. Dana Foundation and Dana-Farber Cancer Institute.

Received 28 December 1995; accepted 1 March 1996.

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