Human Molecular Genetics, 1996, Vol. 5, No. 12 **2061–2066**

Linkage of the gene for the triple A syndrome to chromosome 12q13 near the type II keratin gene cluster

Angela Weber*, Thomas F. Wienker[1], Martin Jung[1], Douglas Easton[2], Heather J. Dean[3], Claudine Heinrichs[4], Andre Reis[1,5] and Adrian J. L. Clark[6]

Department of Paediatrics, University Hospital Dresden, 01307 Dresden, Germany, [1]Microsatellite Centre, Max Delbrück Center (MDC) for Molecular Medicine, 13122 Berlin-Buch, Germany, [2]Department of Community Medicine, Institute of Public Health, University of Cambridge, Cambridge CB2 2SR, UK, [3]Department of Pediatrics, University of Manitoba, Winnipeg, Manitoba R3A 0Z2, Canada, [4]Children's Hospital Queen Fabiola, Free University of Brussels, Brussels, Belgium, [5]Institute of Human Genetics, Humboldt University, 13353 Berlin, Germany and [6]Department of Chemical Endocrinology, St Bartholomew's Hospital, London EC1A 7BE, UK

Received July 17, 1996; Revised and Accepted September 10, 1996

The triple A or Allgrove's syndrome (MIM*231550) is an autosomal recessive disease characterized by the triad of adrenocorticotropic hormone (ACTH) resistant adrenal insufficiency, achalasia and alacrima. Since its first description by Allgrove *et al.* (1978) more than 70 cases from all over the world have been reported. The syndrome manifests itself during the first decade of life with severe hypoglycaemic episodes which can cause sudden death. The frequent association with neurological disorders presenting as a mixed pattern of upper and lower motor neuropathy, sensory impairment, autonomic neuropathy and mental retardation may result in a severely disabling disease. As an additional feature some patients have hyperkeratosis of their palms and soles. We have performed a systematic genome linkage scan in eight triple A families of which three were consanguineous [including the large highly inbred kindred described by Moore *et al.* (1991)]. We obtained conclusive evidence for linkage of the triple A syndrome locus to markers on chromosome 12q13 (*D12S368*, $\theta_{max} = 0$, $Z_{max} = 10.81$) with no indication of genetic heterogeneity. Haplotype and multipoint analyses suggest that the gene is located on a chromosomal segment flanked by the markers *D12S1629* and *D12S312* which are 6 cM apart. This region harbors the type II keratin gene cluster, and potential candidate genes include *SCN8A* and *HOXC* genes.

INTRODUCTION

The triple A syndrome is a potentially lethal disease which includes the combination of adrenal insufficiency, achalasia of the cardia and alacrima (absence of tears) associated with progressive neurological and autonomic dysfunctions (1,3,4). The majority of patients have isolated glucocorticoid deficiency, but in occasional cases mineralocorticoid production is also impaired (5). These clinical symptoms distinguish the triple A syndrome from familial glucocorticoid deficiency, in which ACTH resistant glucocorticoid deficiency is the sole feature caused in some cases by mutations within the ACTH receptor gene (6,7). In addition, the combination of achalasia and alacrima without adrenal failure has been defined as a distinct entity (MIM 200440) (8,9). In the triple A syndrome there is a considerable inter- and intra-familial variability in the occurrence and nature of the neurological and autonomic defects (2,5). Mental retardation and in some cases intellectual deterioration may represent a primary feature of the genetic defect but may also occur secondary to recurrent hypoglycaemia. Chromosomal abnormalities have not been described so far.

We performed linkage analysis to localize the gene for triple A syndrome, and in initial studies excluded localization to two proposed candidate genes, the ACTH receptor and vasoactive intestinal peptide (*VIP*) gene (10). We also excluded the genes for the VIP-1 receptor (*VIP1R*) on chromosome 3p22, for pituitary adenylate cyclase activating peptide (*PACAP*) on chromosome 18p11 which exerts diverse functions as a neuromodulator or neurotransmitter in the central and peripheral nervous systems (11), and for neurotrophin-3 (*NTF3*) on chromosome 12p13 as the causative gene for the triple A syndrome (data not shown).

In the present study, we report the chromosomal localization of the gene responsible for the triple A syndrome to chromosome 12q13 using a genome-wide linkage scan in eight triple A families of Métis Canadian, White European, Australian, Northern American and Moroccan origin.

RESULTS

Two point linkage analysis

As a first step, we screened for linkage using only the highly inbred pedigree 1 of Métis Canadian origin (34 individuals, eight affecteds). Only markers on chromosome 12 suggested linkage to the triple A phenotype; a maximum pairwise lod score of 2.13 was obtained with marker *D12S355* at a recombination fraction $\theta = 0.05$. All eight families ($n = 74$) were then used to test for

*To whom correspondence should be addressed

linkage with additional closely spaced markers from this region on chromosome 12q13 (12). The two-point lod scores of these markers are summarized in Table 3. With marker *D12S368* a maximum lod score of 10.81 at a recombination fraction of $\theta = 0$ was obtained. The cosegregating segment was defined by the two polymorphic markers *D12S368* and *D12S1586* which are ~4 cM apart. There was no evidence of genetic heterogeneity, and all families tested showed linkage with markers from this segment. There were no other regions of suggestive linkage throughout the genome. All patients deriving from families with explicit consanguinity (three pedigrees, 11 affecteds) were investigated for haplotype sharing identical by descent (IBD), and were found to be homozygous for all tested markers between *D12S368* and *D12S1586* (Table 4). For these markers all patients from the four different branches of the Métis Canadian family (PED 1A–D) share a common haplotype. These facts provide strong additional evidence that this region contains the triple A gene.

Haplotype and multipoint linkage analyses

In order to define the smallest interval containing the triple A locus we analyzed all families for recombination events by haplotype reconstruction. Relevant obligatory recombination events could be identified between the triple A locus and marker loci in three pedigrees (Figs 1 and 2). The recombination event in individual 28 of PED 1C (Figs 1a and 2) places the disease locus proximal to *D12S312*, and possibly to *D12S1586* which is not informative. The critical proximal crossing over is in individual 073 of PED 5 (Figs 1b and 2), and places the triple A gene distal to *D12S1629*. Additional recombination events in PED 2 (individuals 038 and 039; Figs 1c and 2) support the assignment of the triple A locus to this interval. From the consanguineous families (PED 1, 3 and 8) further recombination events can be inferred from regions of homozygosity (pedigrees not shown). Individual 065 is a single affected child in PED 3 and shows homozygosity up to and including *D12S368*. In PED 1B, individual 020 is homozygous up to *D12S1629*; other affected individuals from PED 1 show a more extended region of homozygosity. The two affected children 053 and 057 in PED 8 are both homozygous down to and including *D12S312*, thus supporting the distal border (Table 4 and Fig. 2; data for individual 057 not shown).

Table 3. Lod scores at standard recombination rates and at the maximum likelihood estimate of the recombination fraction (Z_{max} and θ_{max}, equal recombination rates in both sexes), for selected marker loci from the pericentromeric region and the proximal long arm of chromosome 12

Marker	LOC	*n*	Het	0.00	0.01	0.05	0.10	0.20	0.30	θ_{max}	Z_{max}
D12S87	57	7	0.68	–∞	–4.36	–0.36	0.80	1.19	0.92	0.23	1.292
D12S85	67	8	0.78	–∞	–1.05	1.96	2.49	1.94	1.02	0.11	2.556
D12S361	71	8	0.54	–∞	–0.76	1.29	1.68	1.32	0.69	0.08	1.815
D12S1629	72	9	0.69	–∞	4.68	5.10	4.53	2.95	1.52	0.03	5.230
D12S368	**73**	**7**	**0.79**	**10.81**	**10.48**	**9.21**	**7.68**	**4.87**	**2.61**	**0**	**10.805**
D12S96	**76**	**7**	**0.57**	**6.97**	**6.77**	**5.96**	**4.92**	**3.01**	**1.54**	**0**	**6.972**
D12S398		**7**	**0.79**	**8.84**	**8.57**	**7.49**	**6.19**	**3.84**	**1.99**	**0**	**8.841**
D12S359	**76**	**7**	**0.82**	**10.12**	**9.79**	**8.52**	**7.07**	**4.56**	**2.46**	**0**	**10.119**
D12S1604	**76**	**8**	**0.78**	**8.86**	**8.54**	**7.32**	**5.90**	**3.54**	**1.76**	**0**	**8.860**
D12S325	**76**	**4**	**0.36**	**5.37**	**5.22**	**4.59**	**3.82**	**2.39**	**1.25**	**0**	**5.373**
D12S1586	**77**	**9**	**0.73**	**9.39**	**9.07**	**7.85**	**6.42**	**3.99**	**2.10**	**0**	**9.389**
D12S312	78	5	0.64	6.17	6.90	6.44	5.40	3.29	1.61	0.02	0.534
D12S1707	78	5	0.55	–∞	3.08	3.55	3.14	2.08	1.13	0.04	3.629
D12S90	80	8	0.70	–∞	2.46	3.67	3.49	2.37	1.19	0.04	3.740
D12S83	84	6	0.72	–∞	–3.73	–0.43	0.47	0.80	0.59	0.25	0.844
D12S92	94	8	0.78	–∞	–6.99	–2.19	–0.70	0.04	0.09	0.32	0.109

Genetic coordinates in cM according to the Généthon map (28) are in the column LOC. *n* gives the total number of different alleles encountered in the pedigree set, Het the estimated heterozygosity value (27). Marker loci showing no recombination with the triple A trait are in bold face, and represent the cosegregating segment.

Table 4. Marker genotypes of affected individuals in terms of size (bp) of allelic PCR products for selected markers of and around the cosegregating segment

Marker	Consanguineous families			Non-consanguineous families				
	PED 1	PED 3	PED 8	PED 2	PED 4	PED 5	PED 6	PED 7
D12S1629	166/166	166/168	166/166	176/166	166/166	166/176	168/168	156/166
D12S368	**204/204**	**204/204**	**200/200**	**206/204**	**204/204**	**206/212**	**204/204**	**204/210**
D12S96	**201/201**	**201/201**	**201/201**	**215/201**	**201/215**	**?/?**	**201/201**	**201/219**
D12S398	**138/138**	**128/128**	**128/128**	**128/128**	**126/130**	**130/134**	**118/118**	**128/128**
D12S359	**215/215**	**215/215**	**209/209**	**219/217**	**217/219**	**221/221**	**?/?**	**221/209**
D12S1604	**280/280**	**280/280**	**268/268**	**280/274**	**274/280**	**274/282**	**268/268**	**274/268**
D12S325	**211/211**	**211/211**	**211/211**	**227/227**	**211/227**	**211/211**	**211/211**	**211/211**
D12S1586	**167/167**	**169/169**	**173/173**	**163/157**	**157/171**	**165/167**	**169/169**	**175/167**
D12S312	245/247	245/245	249/249	247/247	245/247	247/247	249/249	247/249
D12S1707	272/272	272/272	272/274	272/276	272/276	272/272	268/276	278/276

Markers showing no recombination are in bold face.

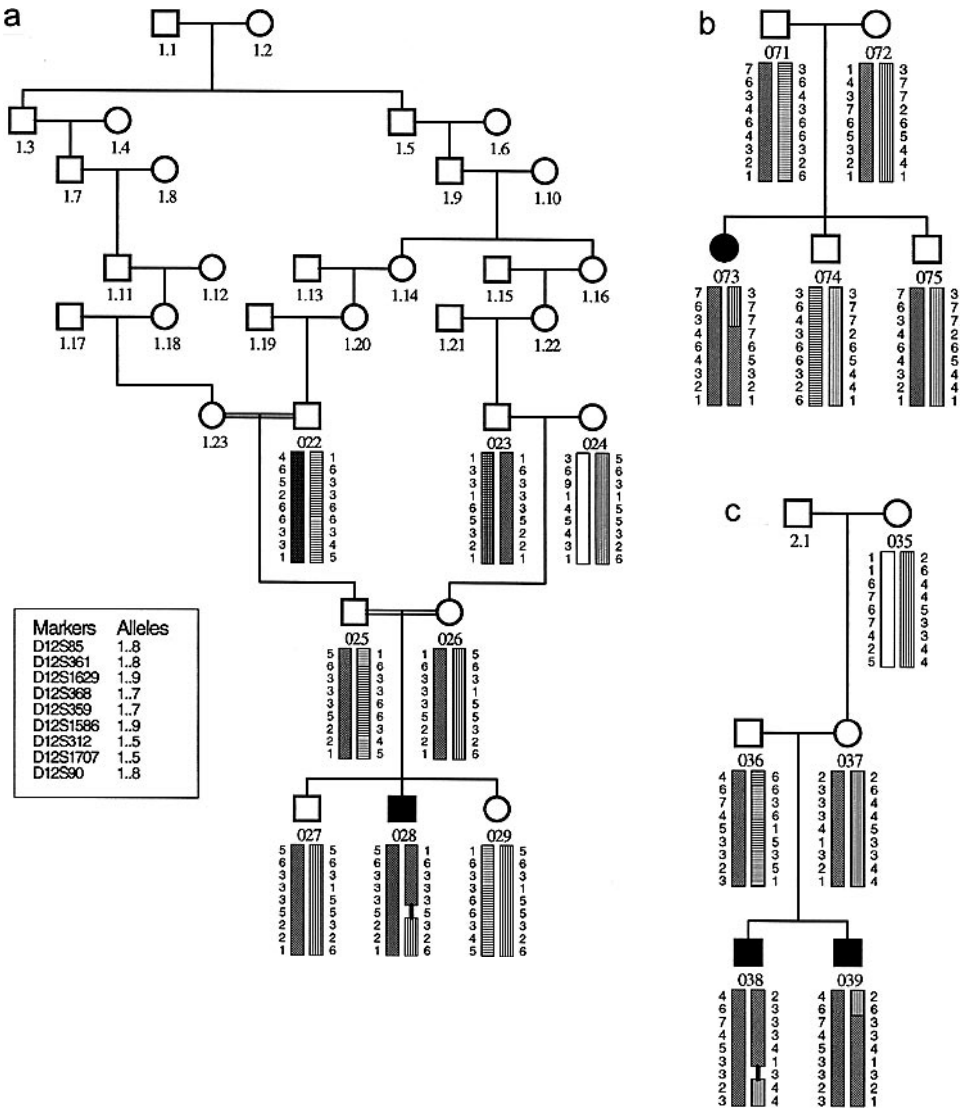

Figure 1. Pedigrees and segregating haplotypes of families 1C (**a**), 5 (**b**) and 2 (**c**) for chromosome 12 markers listed in the box. Squares and circles symbolize males and females, respectively. Black and white symbols indicate affecteds and unaffecteds, respectively. Differently shaded bars indicate segregating chromosomal segments and show regions of crossover. Non informative regions adjacent to critical recombination events are shown by a vertical line.

In the eight families from all over the world we found a total of 12 different marker haplotypes as defined by seven markers tested in the cosegregating segment (Table 4). In contrast to the pedigrees with explicit consanguinity, for PED 6 of Italian origin no consanguinity was reported. However, there is clear homozygosity of markers from the cosegregating segment and beyond. The other pedigrees with no explicit consanguinity apparently represent compound heterozygotes, at least at the marker haplotype level, and probably at the triple A gene, too.

Multipoint linkage analysis using nine markers yielded a maximum multipoint lod score of 13.18 at *D12S368* placing the triple A locus in the 6 cM interval between *D12S1629* and *D12S312* (Fig. 3). *D12S312* and *D12S1707* share a genetic position (13) and are not separated in our data. Physical data are conflicting. In the 'Whitehead map' (14) *D12S312* is clearly centromeric to *D12S1707*, while in the map published by Krauter *et al.* (15) *D12S312* is distal. However, the proximal location seems to be better supported.

DISCUSSION

The combination of linkage analysis and haplotype reconstruction allowed us to position the triple A gene on chromosome 12q13. The current interval is 6 cM and is flanked by *D12S1629* and *D12S312*. The excellent integrated maps available through the Internet (14) with the increasing number of mapped genes of known function and ESTs makes a positional candidate gene approach feasible and attractive. However, the peculiar combination of the triple A symptoms makes a candidate approach very difficult. The involvement of the central, peripheral and autonomic nervous systems as well as the endocrine system and the progressive course of the disease in some patients is suggestive of a defective neuromodulator or neurotrophic factor showing developmental and tissue-specific patterns of expression. On the other hand, the degenerative nature of the neurological features, and in some reports the adrenal disorder, is more suggestive of a cumulative toxic effect analogous to that seen in adrenoleukodystrophy resulting from

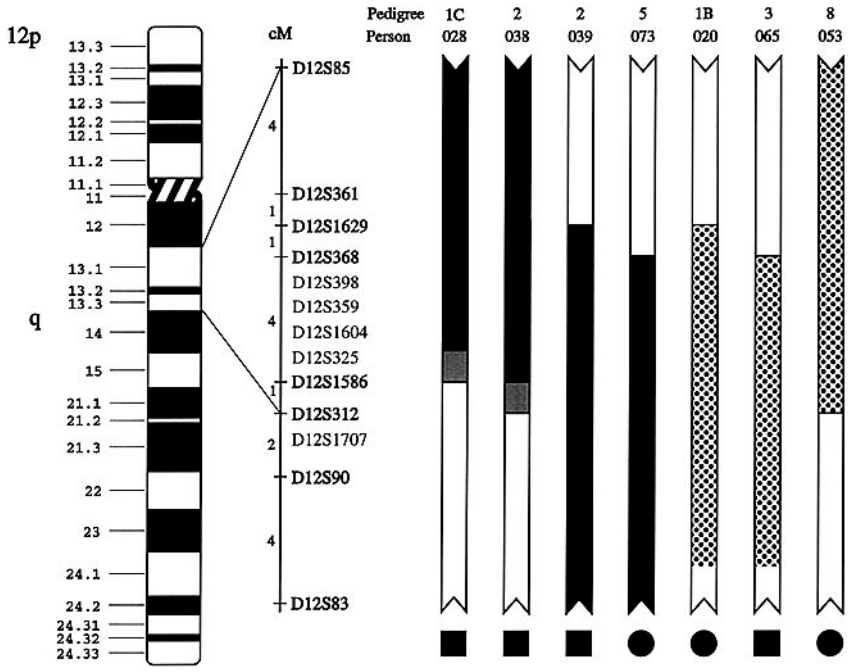

Figure 2. Schematic presentation of chromosome 12 with details of the genetic map and selected chromosomal segments representing key recombination events. The idiogram is at the 550 band level resolution according to ISCN 1995 (33). The genetic map is constructed using the Généthon data (28), and the correlation of physical and genetic locations is according to Bray-Ward *et al.* (12). Marker loci with a distinctive coordinate position are in bold face; markers sharing one position are in light face (see column LOC of Table 1). Dark chromosomal segments indicate the obligatory position of the triple A locus inferred from the particular pedigree and individual; grey areas are possible locations. Dotted areas represent regions of homozygosity in haplotypes of affected individuals from three pedigrees with explicit consanguinity.

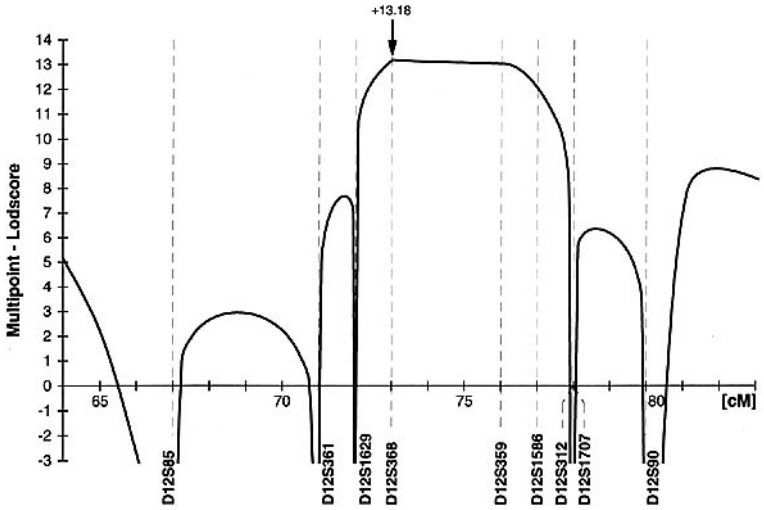

Figure 3. Multipoint lod scores between the triple A gene and markers *D12S85, D12S361, D12S1629, D12S368, D12S359, D12S1586, D12S312, D12S1707* and *D12S90*.The X-axis indicates the genetic location coordinate according to the Généthon map (28); the zero coordinate is at the short arm telomere (12pter).

defects in peroxisomal membrane protein (16). Alternatively, a mutation in a transcription factor for genes involved in the neuroendocrine system could be envisaged, thus being responsible for the apparent pleiotropic gene action.

There are several known genes mapped to the critical region on 12q13: nine keratin genes *(KRT1, -2, -4, -5, -6A, -6B, -7, -8* and *-18)*, the genes for beta-7 integrin (*ITGB7)*, retinoic acid receptor γ (*RARG*), *trans*-acting transcription factor (*SP1*) and the homeobox C gene cluster (*HOXC@)* (15,17). None of the genes mentioned are strong candidates which could explain the entire triple A phenotype. However, the close vicinity to the type II keratin (*KRT*) gene cluster raises the possibility of a contiguous gene syndrome as some triple A patients suffer from hyperkeratosis of the palms and soles with fine palmar creases (1,5,18). In addition, by fluorescence *in situ* hybridization the human homologue of the mouse *Scn8a* gene, encoding a voltage-

gated sodium channel type VIII, was mapped to chromosome 12q13 (19). Loss of expression of the mouse *Scn8a* gene led to a neurological disorder with features of muscular atrophy, ataxia and juvenile lethality (motor endplate disease, *med*). It was predicted that the human homologue of *Scn8a* is a candidate gene for inherited neurogenerative disease (19). Cloning and fine mapping of human *SCN8A* is necessary to determine if it maps to the triple A critical region.

Strategies for the identification of the gene responsible for the triple A syndrome may include molecular cytogenetic analysis focusing on the cosegregating segment on chromosome 12q13 and positional and functional candidate approaches (20). Preliminary cytogenetic analysis of several patients from different pedigrees did not reveal any gross chromosomal aberration, and in particular no visible rearrangements of chromosome 12 (data not shown).

Our finding that the triple A gene resides between *D12S1629* and *D12S312* represents the first step towards the isolation and cloning of the triple A gene. The eventual identification of the causative gene may reveal novel aspects of cell signalling and neurodevelopment as well as of the regulation of adrenocortical function. Understanding the molecular defect of the triple A syndrome may allow a presymptomatic testing of neonates in affected families, and may permit the rational design of therapies in this condition.

MATERIALS AND METHODS

Family material

We obtained DNA samples from eight families from six countries (PED 1 was of Métis Canadian, PED 2–7 of White European and PED 8 of Moroccan origin) including 19 triple A patients (13 males and six females) and 55 unaffected family members (pedigrees available upon request). Métis people are a distinct cultural aboriginal group in Canada who descended from North American Indians and French Canadian settlers. Three families are consanguineous (PED 1, 3 and 8), and five families have two or more affected individuals (PED 1, 2, 6, 7 and 8). The diagnosis of triple A syndrome was made on the basis of clinical and biochemical evidence of glucocorticoid, and in some cases mineralocorticoid deficiency in combination with deficient tear production in a Schirmer test and a pathological oesophageal motility visible on contrast radiography using barium sulphate providing evidence for achalasia. In addition, a variety of neurological symptoms have been found in these patients, being previously described in six of the eight families (1–5,21). Amongst our families is the large and highly inbred Métis Canadian kindred described by Moore *et al.* (2) living in a remote area north of Winnipeg in Canada, and we were able to examine the eight affected children and to obtain further pedigree information. This family has an explicit genealogy extending over nine generations with multiple consanguinity loops.

DNA-marker analysis

Microsatellite analysis was performed with fluorescently labelled primers by standard semi-automated methods (22) using the ABI 373 and 377 DNA Sequencers. Primers for the 10 µl PCR reactions were obtained from the UK MRC Human Genome Mapping Project primer set established by John Todd or from Oswell Scientific (Guildford, UK), from the human linkage map generated by Généthon (23), and from the MDC microsatellite mapping set (Jung,M., unpublished results). The total genome scan comprises 345 marker loci spaced at ~10 cM throughout the autosomal part of the genome. The majority of markers are dinucleotide repeat loci. PCR reactions were performed according to the manufacturer's protocol using Hybaid-OmniGene (Hybaid, UK) or PCR 9600 thermocyclers (Applied Biosystems). Data were exported as a text file from Genotyper 1.0 program for subsequent linkage analysis after allele calling was checked by experienced operators.

Linkage analysis

Prior to linkage analysis, expected lod scores were determined by simulation studies using the SLINK program (24). The large Métis pedigree poses formidable computational problems due to multiple consanguinity loops which span up to six generations, and hence was broken down into four parts (PED 1A, B, C and D). Multiple loops were taken into account where necessary in order not to force the flow of a disease allele through a particular branch of the pedigree (PED 1C, Fig. 1a). For the complete pedigree set of all eight families an expected lod score of +5.15 was obtained under the following assumptions: (i) a regular, fully penetrant autosomal recessive trait locus with disease allele frequency of 0.002, (ii) no heterogeneity, (iii) a codominant marker locus of four alleles and uniformly distributed allele frequencies (heterozygosity value 0.75), and (iv) a recombination rate of 0.05. Five hundred replicates took ~22 h runtime on a powerful PC. In the same way a screening subset of the pedigree material was designed and tested yielding an expected lod score of +4.57, which ran much faster. The expected resolving power of the study was estimated according to Boehnke (25). Simulation studies using the full pedigree set and the genetic model as given above resulted in a maximum expected lod score of +10.33 at a recombination fraction of zero. This was the case only for markers from the centromeric region of chromosome 12. Two-point linkage analysis was performed using the programs MLINK and ILINK of the LINKAGE package version 5.2 (26), and the FASTLINK modifications, respectively (27). Marker allele frequencies were assumed to be uniformly distributed, since no reliable estimates can be obtained with pedigree material from populations as diverse as those in this study. However, the number of alleles encountered and estimates of heterozygosity values (28) are given in Table 3. These values fit remarkably well with those given by Généthon (13). Multipoint linkage analysis was performed using FASTLINKMAP (27) with five marker loci in each run and 'downcoding' of alleles in each pedigree (29). The map (Fig. 3) was constructed from several runs with overlapping sets of marker loci. Disregarding consanguinity and eventual biparental grandparents we used the VITESSE program (30) in addition with qualitatively similar results. However, lod scores were considerably lower. There was no indication of locus heterogeneity among the pedigrees studied, and accordingly a heterogeneity test (A-test, HOMOG programm; 29) gave insignificant results. Haplotype analysis was used for error elimination during the linkage scan and for the determination of the critical linkage segment. Haplotype construction was performed using the CRIMAP program with the CHROMPIC option (31), and with the aid of the CYRILLIC program version 2.0 (32) (Fig. 1a–c).

ACKNOWLEDGEMENTS

We appreciate the cooperation of all triple A families involved, and the excellent information delivered by their physicians. We thank B. Bayer and S. Lowe for technical assistance, G. Nürnberg for help with illustrations, and F. Rüschendorf for the CRIMAP calculations. We gratefully acknowledge C. Greenberg for her support during the collection and preparation of the Métis-Canadian DNA samples, M. M. Town and C. G. Mathew for aliquots of some fluorescently labelled primers. A.W. was supported by a Wellcome Travelling Research Fellowship (042570) and a Wellcome Research Travel Grant. This study was supported by the Medical Research Council (UK) by providing the UK HGMP primer set. The Microsatellite Centre is supported by the Bundesministerium für Bildung und Forschung.

ABBREVIATIONS

ACTH, adrenocorticotrophic hormone; bp, base pair; cM, centimorgan; EST, expressed sequence tags; ISCN, International System for human Cytogenetic Nomenclature; PCR, polymerase chain reaction; PED, pedigree.

REFERENCES

1. Allgrove,J., Clayden,G.S., Grant,D.B. and Macaulay,J.C. (1978) Familial glucocorticoid deficiency with achalasia of the cardia and deficient tear production. *Lancet*, **i**, 1284–1286.
2. Moore,P.S.J., Couch,R.M., Perry,Y.S., Shuckett,E.P. and Winter,J.S.D. (1991) Allgrove syndrome: an autosomal recessive syndrome of ACTH insensitivity, achalasia and alacrima. *Clin. Endocrinol.*, **34**, 107–114.
3. Grant,D.B., Dunger,D.B., Smith,I. and Hyland,K. (1992) Familial glucocorticoid deficiency with achalasia of the cardia, associated with mixed neuropathy, long tract degeneration and mild dementia. *Eur. J. Pediatr.*, **1**, 85–89.
4. Heinrichs,C., Tsigos,C., Deschepper,J., Drews,R., Collu,R., Dugardeyn,C., Goyens,P., Ghanem,G.E., Bosson,D., Chrousos,G.P. and Van Vliet,G. (1995) Familial adrenocorticotropin unresponsiveness associated with alacrima and achalasia: biochemical and molecular studies in two siblings with clinical heterogeneity. *Eur. J. Pediatr.*, **154**, 18–23.
5. Grant,D.B., Barnes,N.D., Dumic,M., Ginalska-Malinowska,M., Milla,P.J., von Petrykowski,W., Rowlatt,R.J., Steendijk,R., Wales,J.H.K. and Werder,E. (1993) Neurological and adrenal dysfunction in the adrenal insufficiency/alacrima/achalasia (3A) syndrome. *Arch. Dis. Child.*, **68**, 779–782.
6. Clark,A.J.L., McLoughlin,L. and Grossman,A. (1993) Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. *Lancet*, **341**, 461–462.
7. Weber,A. and Clark,A.J.L. (1994) Mutations of the ACTH receptor gene are only one cause of familial glucocorticoid deficiency. *Hum. Mol. Genet.*, **3**, 585–588.
8. Nussinson,E., Hager,H., Samara,M., Bar-Nathan,N. and Siplovich,L. (1988) Familial achalasia with absent tear production. *J. Pediatr. Gastroenterol. Nutr.*, **7**, 284–287.
9. Haverkamp,F., Zerres,K. and Rosskamp,R. (1989) Three sibs with achalasia and alacrimia: a separate entity different from triple-A syndrome. *Am. J. Med. Genet.*, **34**, 289–291.
10. Weber,A., Farrall,M., Dean,H.J. and Clark,A.J.L. (1995) Exclusion of the ACTH receptor and vasoactive intestinal peptide loci as candidate genes for the triple A syndrome. *Horm. Res.*, **44** [Suppl. 1], 5.
11. Arimura,A. (1992) Pituitary adenylate cyclase activating polypeptide (PACAP): discovery and current status of research. *Regul. Pept.*, **37**, 287–303.
12. Bray-Ward,P., Menninger,J., Lieman,J., Desai,T., Mokady,N., Banks,A. and Ward,D.C. (1996) Integration of the cytogenetic, genetic, and physical maps of the human genome by FISH mapping of CEPH YAC clones. *Genomics*, **32**, 1–14.
13. Dib,C., Fauré,S., Fizames,C., Samson,D., Drouot,N., Vignal,A., Millasseau,P., Marc,S., Hazan,J., Seboun,E., Lathrop,M., Gyapay,G., Morissette,J. and Weissenbach,J. (1996) A comprehensive genetic map of the human genome based on 5264 microsatellites. *Nature*, **380**, 152–154.
14. Hudson,T.J., Stein, L.D., Gerety,S.S., Ma,J., Castle,A.B., Silva,J., Slonim,D.K., Babtista,R., Kruglyak,L., Xu,S.H., *et al.* (1995) An STS-based map of the human genome. *Science*, **270**, 1945–1954.
15. Krauter,K., Montgomery,K., Yoon,S-J., LeBlanc-Straceski,J., Renault,B., Marondel,I., Herdman,V., Cupelli,L., Banks,A., Lieman,J., Menninger,J., Bray-Ward,P., Nadkarni,P., Weissenbach,J., Le Paslier,D., Rigault,P., Chumakov,I., Cohen,D., Miller,P., Ward,D. and Kucherlapati R. (1995) A second-generation YAC contig map of human chromosome 12. *Nature*, **377** [Suppl.], 321–334.
16. Mosser,J., Douar,A-M., Sarde,C.-O., Kioschis,P., Feil,R., Moser,H., Poustka,A.-M., Mandel,J.-L. and Aubourg,P. (1993) Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature*, **361**, 726–730.
17. Acampora,D., D'Esposito,M., Faiella,A., Pannese,M., Migliaccio,E., Monelli,F., Stornaiuolo,A., Nigro,V., Simeone,A. and Boncinelli,E. (1989) The human HOX gene family. *Nucleic Acids Res.*, **17**, 10385–10402.
18. Counahan,R. and West,R. (1974) Ocular and fingertip abnormalities in isolated glucocorticoid deficiency. *J. Pediatr.*, **85**, 580–581.
19. Burgess,D.L., Johrman,D.C., Galt,J., Plummer,N.W., Jones,J.M., Spear,B. and Meisler,M.H. (1995) Mutation of a new sodium channel gene, Scn8a, in the mouse mutant 'motor endplate disease'. *Nature Genet.*, **10**, 461–465.
20. Collins,F.S. (1995) Positional cloning moves from perditional to traditional. *Nature Genet.*, **9**, 347–350.
21. Esposti,A.D., Ambrosioni,G., Giardina,A., Giovannini,G., Laghi,M.G., Molinari,P.P., Salvatori,P. and Zaniboni,M.G. (1985) Sindrome familiare di ipoglicocorticismo da insensibilatà all 'ACTH, acalasia, alacrimia, con associata neuromiopatia distale. *Minerva Pediatr.*, 37, 353–359.
22. Reed,P.W., Davies,J.L., Copeman,J.B., Bennett,S.T., Palmer,S.M., Pritchard,L.E., Gough,S.C.L., Kawaguchi,Y., Cordell,H.J., Balfour,K.M., Jenkins,S.C., Powell,E.E., Vignal,A. and Todd,J.A. (1994) Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. *Nature Genet.*, **7**, 390–395.
23. Gyapay,G., Morissette,J., Vignal,A., Dib,C., Fizames,C., Millasseau,P., Marc,S., Bernardi,G., Lathrop,M. and Weissenbach,J. (1994) The 1993–94 Généthon human genetic linkage map. *Nature Genet.*, **7**, 246–339.
24. Weeks,D.E., Ott,J. and Lathrop,G.M. (1990) SLINK: a general simulation program for linkage analysis. *Am. J. Hum. Genet.*, **47**, A204.
25. Boehnke,M. (1994) Limits of resolution of genetic linkage studies: implications for the positional cloning of human disease genes. *Am. J. Hum. Genet.*, **55**, 379–390.
26. Lathrop,G.M., Lalouel,J.M., Julier,C. and Ott,J. (1984) Strategies for multilocus linkage analysis in humans. *Proc. Natl. Acad. Sci. USA.*, **81**, 3443–3446.
27. Cottingham,R.W.Jr., Idury,R.M. and Schaeffer,A.A. (1993) Faster sequential genetic linkage computations. *Am. J. Hum. Genet.*, **53**, 252–263.
28. Boehnke,M. (1991) Allele frequency estimation from data on relatives. *Am. J. Hum. Genet.*, **48**, 22–25.
29. Ott,J. (1991) *Analysis of Human Genetic Linkage*. Rev. ed., John Hopkins University Press.
30. O'Connell,J.R. and Weeks,D.E. (1995) The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. *Nature Genet.*, **11**, 402–408.
31. Lander,E.S. and Green,P. (1987) Construction of multilocus genetic linkage maps in humans. *Proc. Natl. Acad. Sci. USA.*, **84**, 2363–2367.
32. Chapman,C. (1995) *Cyrillic 2 for pedigree drawing.* Cherwell Scientific, Oxford.
33. ISCN (1995): An international system for human cytogenetic nomenclature. Mitelman,F. (ed.), S.Karger, Basel.