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Autosomal dominant adult neuronal ceroid lipofuscinosis

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Chapter 4

AD-ANCL

Genetics

Genetic analysis of a family with autosomal dominant neuronal ceroid lipofuscinosis

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Abstract

Objective: to identify the genetic defect in a family with autosomal dominant adult neuronal ceroid lipofuscinosis.

Methods: We applied genome wide STR-based linkage analysis to a Dutch multigenerational family with autosomal dominant ANCL, and performed subsequent fine-mapping of candidate loci.

Results: Under an autosomal dominant model 8 loci were suggestive for linkage with a LOD-score > 1 . Three of these loci were most promising: two subsequent markers gave a LOD-score above 1 (on chr. 2 and chr. 21) ; multipoint LOD-score calculations achieved a maximum score of 1.96 on chr. 12. These 3 loci were further analyzed by genotyping with additional STR markers in the region. In the 3 loci a disease haplotype appeared to segregate with the disease. Markers in the proximity of the known NCL loci (1p32, 11p15, 16p12.1, 13q21, 15q21-33, 8p23, 11p15.5) were checked, but none passed a LOD-score value of 1.

Conclusion: linkage analysis in this small family with autosomal dominant ANCL identified 3 genetic loci which segregate with the disease, but further studies are needed.

Introduction

Although the gene symbol CLN4 has been assigned to adult NCL, no genetic defects are known in adult forms as yet. Unlike childhood NCL forms which are inherited in an autosomal recessive mode ¹⁻³, the genetic defects in adult NCL are most likely heterogeneous: adult NCL occurs sporadic or familial with recessive inheritance (Kufs' disease), but also with an autosomal dominant mode of inheritance in some families, called Parry disease ^{4 5 6 7 8 9}.

Our Dutch dominant ANCL family has been the subject of previous reports, concerning clinical ⁸, pathological ¹⁰, neurophysiological ¹¹, auditory (chapter 6) and visual aspects (chapter 7).

Here we report on genome-wide linkage analysis of this family.

Methods and patients

We analysed a multigenerational family with autosomal dominant ANCL, with 6 known affected individuals in 3 generations. Blood samples for DNA extraction were obtained from 22 individuals including 5 patients, 13 healthy at risk individuals and 4 spouses. The family pedigree is shown in fig.1 . Genome-wide linkage analysis was performed, using 400 STR markers, with an average distance of 8 cM, and with subsequent fine-mapping of candidate loci.

Family characteristics:

Patient 1

This woman with generalized tonic clonic seizures from the age of 44 years, had progressive cognitive decline, myoclonus and parkinsonism. She died at the age of 51.

Patient 2

This woman is a daughter of patient 1. She had myoclonus of arms and face from the age of 46 years, followed by progressive dementia, parkinsonism, generalized tonic clonic seizures and psychotic episodes. She died at the age of 59 years.

Patient 3

This sister of patient 2 had several depressive episodes, and developed generalized epileptic seizures at the age of 42 years, followed by psychotic episodes, parkinsonism, myoclonus and progressive dementia. She died at the age of 56 years.

Patient 4

This brother of patients 2 and 3 had myoclonic jerks of the arms since the age of 36 years. He had progressive memory impairment, depressive episodes, parkinsonism, facial dyskinesias and generalized tonic clonic epileptic insults. He died at the age of 56 years.

Patient 5

This daughter of patient 2 had tension-type headache and migraine attacks without aura from the age of 19 years. Since the age of 32, she has had progressive myoclonus and slight memory difficulties, and epilepsy.

Patient 6

This son of patient 4 has had myoclonus of the thumb and arms from the age of 25, and frequent tonic clonic seizures since age 31. He has severely decreased visual acuity, and moderate parkinsonism.

Genome-wide linkage analysis

A genome-wide scan was performed by use of multiplex mapping panel of 400 autosomal STR markers with an average inter-marker distance of 8 cM.

Genomic DNA was PCR amplified in 26 multiplex reactions using fluorescently labeled primers. PCR products were resolved on an ABI3730 automated sequencer (Applied Biosystems). Genotypes were assigned using in-house developed genotyping software. Two-point and multi-point LOD-scores were calculated using MLINK and LINKMAP from the LINKAGE software package version 5.2 (Lathrop et al. 1985). We assumed an autosomal dominant inheritance model with reduced age-dependent penetrance for the trait locus. The estimated population frequency of the disease gene was set at 0.00001. Nine liability classes for disease penetrance were used, based on the cumulative risk curve calculated from the mean onset age for ANCL in the family, with a maximal disease penetrance of 100% when ≥ 60 years. Phenocopy rates were also age-dependent.

Results

Genome-wide STR-based linkage analysis

In an 8 cM density genome-wide scan, we calculated 2-point LOD-scores ≥ 1 for 10 STR markers in 8 distinct chromosomal regions (table 1). On chromosomes 2 and 21 two subsequent markers achieved a 2-point LOD-score above ≥ 1 . A maximum 2-point LOD-score of 1.647 was reached at the chromosome 21. Multipoint linkage analysis of the 8 loci resulted in a maximum score of 1.731, for the chromosome 12 locus. Markers in the proximity of the known NCL loci (1p32, 11p15, 16p12.1, 13q21, 15q21-33, 8p23, 11p15.5) were

checked but none passed a LOD-score value of 1. Three loci were prioritized for further examination. The loci on chromosomes 2 and 21 were selected because two subsequent markers showed suggestive linkage, and the chromosome 12 locus was selected because of the maximum multipoint LOD-score. Additional STR markers were selected for genotyping to achieve a marker density of about 1 STR/1 cM/locus.

Fine mapping of candidate loci and sequencing of positional candidate genes

Chromosome 12

For the markers included in the genome-wide scan a maximum multipoint LOD-score of 1.731 was obtained on chromosome 12 at D12S1056 . Twenty-two additional STR markers in the region covering 25 MB were used for genotyping in our family. LOD score calculations achieved a new maximum of 1.962 for 4 markers in the region (Table 2). Segregation analysis defined a risk-haplotype that appeared to segregate with the disease in the family. All patients were carrier of the risk-haplotype and meiotic recombination events in one patient deliniated the candidate region to a 13.11Mb region flanked by markers D12S1655 and GATA194G07. However, the entire risk-haplotype was also observed in one at risk individual. A small part of the risk haplotype at the telomeric end was also present in an individual who has just a 1% probability to still become ill, while meiotic recombination finemaps a priority region from D12S1655 till D12S375.

The potential candidate region on chromosome 12q14-21 contains 95 known genes. Of these, 3 positional candidate genes, FAM19A2 (coding for a gene product which is postulated to function as a brain-

specific chemokine), GNS (glucosamine (N-acetyl)-6-sulfatase) , LYZ (lysozyme), were subjected to mutation analysis but did not reveal any mutations that could explain the disease in this family.

Chromosome 2

Two of the 27 markers tested on chromosome 2 gave a 2-point LOD-score above 1. At two subsequent markers, D2S1360 and D2S144, a LOD-score of 1.332 was calculated. Multi-point LOD-score analyses at this locus achieved a maximum score of 1.333. Following the genome-scan results, 8 additional STR markers were typed for segregation analyses. A minimal risk haplotype of 14.6 Mb was defined in a patient by two meiotic recombinants. The risk haplotype was present in all patients but also in two at risk sibs and one more relative.

Chromosome 21

In the genome-wide scan the highest 2-point LOD score of 1.647 was achieved at marker D21S1893. The adjacent marker D21S1440 also reached a LOD-score above 1.

Four additional STR markers were genotyped in this region yielding a risk-haplotype from D21S11920 till the telomeric end. In addition to the patients parts or the entire risk haplotype was also present in 3 at risk individuals.

Discussion

To our knowledge, this is the first report on genetic analysis of a family with adult NCL, with a pedigree which suggests autosomal dominant inheritance. Although this study was unable to identify a

single genetic defect, three haplotypes were identified which segregate with the disease. However, conclusions are hampered by segregation with some unaffected family members. The study is limited by the small number of affected family members, and also by lack of predictive information in unaffected individuals. Although we showed that an abnormal electroencephalogram in an asymptomatic family member could be predictive of disease ¹¹ , it was not applied in this study because of ethical reasons.

Because of small family size, relatively low LOD scores were expected and obtained from the initial genome-wide STR-based linkage analysis. We used an approach where (from the 8 loci suggestive for linkage with a LOD-score > 1, in an autosomal dominant model) only 3 loci were selected for detailed analysis by genotyping additional STR markers in the region: the locus with the highest multipoint LOD score of 1.73 on chr. 12, and two other loci (on chr. 2 and chr. 21, where two subsequent markers gave a LOD-score above 1). The current findings are therefore insufficient to exclude genetic loci apart from our 3 investigated loci.

It is unlikely that known genetic loci for NCL contribute to the etiology of our family, since markers in the proximity of the known NCL loci (1p32, 11p15, 16p12.1, 13q21, 15q21-33, 8p23, 11p15.5) were checked, while none passed a LOD-score value of 1.

Furthermore, PPT activity was normal in patients of our family ¹⁰, which excludes CLN1. GRODs are present in our patients, but are uncommon in NCL forms other than CLN1.

Besides , our pathological and clinical findings were very similar to other reported AD-ANCL families, which suggests a nosological entity. AD-ANCL is also distinct from other ANCL forms because of its dominant inheritance. It is intriguing that all other NCL forms show

an autosomal recessive mode of inheritance. Dominant occurrence at young age of a devastating disease like NCL would most likely have a very negative effect on the chance of reproductive offspring.

While 8 genes are identified in childhood NCL³, knowledge on the genetic basis of adult NCL is very limited. Our study did not identify a single gene. However, our limited findings may contribute because genetic knowledge on ANCL is so far completely lacking.

Some individual cases with adult presentation of one of the childhood NCL forms have been reported. For example, two sisters with neuronal ceroid lipofuscinosis presenting in the fourth decade had a profound deficiency of PPT related to the CLN1 gene mutation R151X and a novel missense mutation G108R¹². Another ANCL patient was reported, who also was a compound heterozygote with mutation R151X, but here the other mutation was a novel mutation, p.Cys45Tyr, which probably disrupts one of two hydrogen bonds with Asp79,6 causing a less severe structural defect. Enzyme activity of PPT1 was diminished but not abolished¹³. PPT activity was normal in patients of our family ¹⁰.

Sleat et al identified CLN5 as a cause in an ANCL patient, using an innovative proteomic approach, using the presumption that lysosomal storage diseases arise from mutations in genes encoding lysosomal proteins that contain mannose 6-phosphate, a carbohydrate modification that acts as a signal for intracellular targeting to the lysosome. Purification and quantification of mannose 6-phosphorylated proteins by affinity chromatography in 23 patients with confirmed or possible lysosomal disease, identified or validated the genetic basis for disease in 8 cases¹⁴. In one ANCL patient [CABM-BR19], this method indicated loss of CLN5, which confirmed previously identified but unreported missense changes (377G>A (Cys126Tyr) and 1121A>G (Tyr374Cys)).

This group also found an unusual cause in a patient suspected of ANCL (HSB#4165), where this chromatographic method indicated absence of SGSH, which is a lysosomal enzyme that is defective in MPSIIIA, a disorder unrelated to the NCLs. Sequence analysis indicated compound heterozygosity for two missense mutations, Glu355Lys and Ser298Pro in SGSH, both of which are documented pathogenic alleles in MPSIIIA. While clinical details and ultrastructure were not reported in this case, it is unclear whether this case fulfills diagnostic criteria for ANCL, which has often led to misdiagnosis ¹⁵.

This proteomic approach directed towards study of lysosomal enzymes could also be useful in future studies of AD-ANCL. A different approach for further research could be genetic linkage study of multiple families with presumed identical autosomal dominant disease, with similar clinical presentation and ultrastructure. This is now the focus of the Rare NCL Gene Consortium (RNGC), which was set up in 2006 to facilitate identification of all NCL genes ¹⁶.

Figures

STR Marker	Chr.	Mb	cM	theta = 0
D1S2682	1	246.196	288.29	1.143
D2S1360	2	17.355	38.33	1.332
D2S144	2	25.354	45.30	1.332
D7S637	7	154.034	173.03	1.009
GATA114H09	10	106.636	127.11	1.332
D12S1056	12	58.832	75.17	1.165
D15S652	15	90.318	90.02	1.181
ATA67B07	16	1.942	6.08	1.472
D21S1440	21	38.063	36.77	1.109
D21S1893	21	40.278	43.67	1.647

Table 1 : 8cM density genome-wide scan, showing 2-point LOD-scores ≥ 1 for 10 STR markers in 8 distinct chromosomal regions

STR Marker	Mb	0	0.01	0.05
D12S297	50.899	-0.472	-0.157	0.248
D12S398	51.483	-0.472	-0.157	0.248
D12S1586	52.433	1.165	1.139	1.035
D12S104	57.188	-0.013	-0.013	-0.013
D12S1056	58.832	1.165	1.139	1.035
D12S1662	59.131	-1.658	-1.254	-0.733
D12S83	59.176	-1.883	-1.539	-1.003
D12S1655	59.222	-1.641	-1.308	-0.819
D12S1726	60.746	1.926	1.889	1.739
GATA71A08	61.11	1.165	1.139	1.035
D12S1022	61.941	0.511	0.499	0.449
D12S1610	63.291	1.963	1.926	1.775
D12S1649	63.874	1.963	1.926	1.775
D12S1686	63.951	1.963	1.926	1.775
D12S2202	64.059	0.457	0.446	0.403
D12S75	64.784	1.963	1.926	1.775
D12S375	67.231	-0.124	0.297	0.703
GATA134C11	69.409	0.457	0.446	0.401
GATA194G07	72.329	-2.321	-1.775	-0.93
D12S375	73.500	0.456	0.445	0.402
D12S1660	74.749	-4.144	-1.875	-0.724
D12S326	76.498	-4.14	-1.866	-0.722

Table 2: genotyping on chromosome 12 using 22 additional STR markers, covering 25 MB, where LOD score calculations achieved a new maximum of 1.963 for 4 markers in the region.

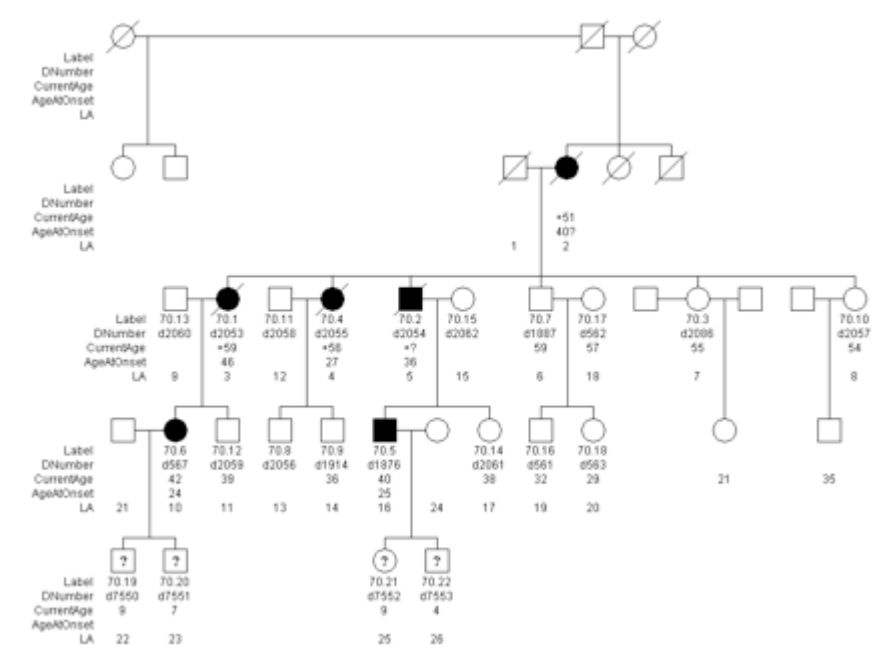


Fig. 1 pedigree

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