Molecular genetics of nocturnal enuresis: clinical and genetic heterogeneity

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Forty-two children with nocturnal enuresis (27 with primary, 4 with secondary nocturnal enuresis and 11 with combined primary nocturnal enuresis and daytime wetting) were selected retrospectively from a study of 167 consecutive children with enuresis. The aim of the study was to collect formal genetic data, perform molecular genetic linkage-analyses with five microsatellite markers on chromosomes 13q, 12q or 8q and specify the associations between genetic findings and clinical, as well as psychiatric diagnoses. Positive linkage of nocturnal enuresis to one of the microsatellite markers was possible in 27 children from 23 families and was not possible in 15 children. Somatic findings in both the groups with and without possible assignment of nocturnal enuresis to a marker were heterogeneous. Psychiatrically, a low rate of behavioural problems was apparent. These findings support the hypothesis of genetic and phenotypical heterogeneity of nocturnal enuresis, without linkage of specific psychiatric and somatic phenotypes to certain chromosome markers.

Formal and molecular genetics, nocturnal enuresis, psychiatry, urology

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Enuresis is one of the most common childhood problems in both paediatrics and child psychiatry. According to epidemiological studies, about 10% of 7-y-old children and 1% of young adults are still bed-wetters (1). Among all forms of nocturnal enuresis and daytime incontinence, primary nocturnal enuresis (PNE) is the most common form. In addition to difficult arousal from sleep (1) despite normal sleep architecture (2, 3) and a variation of the circadian excretion of AVP (arginine vasopressin) (4), monosymptomatic nocturnal enuresis shows the greatest genetic disposition.

In empirical family studies nocturnal enuresis occurred with high frequency in the parents, siblings and other near relatives of bed-wetters (5, 6). The highest incidence of enuresis was in families in which both parents had been enuretic in childhood (77%). The incidence of enuresis if either father or mother had been enuretic was 43 and 44%, respectively.

In Bakwin’s twin-study (7) the concordance rate for monozygotic twins was twice as high as for dizygotic twins. Hallgren’s data (7) showed similar results: of 30 pairs of monozygotic twins, 21 (70%) were concordant, while all 10 pairs of dizygotic twins were discordant. Adoption studies were not performed for enuresis.

In addition to these formal genetic findings, molecular genetic analyses were performed recently in primary nocturnal enuresis. In a Danish study by Eiberg, 11 large families with PNE were identified, in which transmission of enuresis seemed to follow an autosomal dominant mode of inheritance with high penetrance (90%). There was evidence for assignment of PNE to chromosome 13q (13q13–13q14.2) in five of these families, as linkage analyses gave positive lod scores for microsatellite markers D13S291 and D13S263 ($Z = 3.77$ resp. 2.9). The arginine vasopressin gene as candidate-gene (chromosome 20p13) could be excluded (8). In later publications, positive lod scores for D8S264 on chromosome 8q were found in two families, not linked to chromosome 13q. In contrast, linkage to chromosome 12q could not be verified in any of the families (9, 10).

In a Swedish study of 392 children with primary nocturnal enuresis, 48% (187) were considered to be sporadic cases, 9.4% (37) seemed to follow an autosomal recessive and 43% (168) an autosomal dominant mode of inheritance (11, 12). Of the latter, 16 multigenerational families were selected for further linkage studies. While only three families showed a positive lod score for the markers on chromosome 13q, six families were linked to a region restricted by the polymorphic loci D12S368 and D12S101 ($Z_{max} 3.88$ at D12S80). One family showed indications of linkage to loci on both 13q and 12q.

All of these studies demonstrate genetic heterogeneity in nocturnal enuresis with either linkage or exclusion of linkage to one of three different chromosomes (13q, 12q or 8q) in different families. Methodically, the phenotype of nocturnal enuresis is often not described in detail. In addition, the clinical data was often gained retrospectively with the possibility of recollection bias.
The objective of our study with 42 German children with nocturnal enuresis and their families was to describe the phenotype in as great detail as possible, to collect formal genetic data, to perform molecular genetic linkage-analyses of nocturnal enuresis with all five markers on chromosomes 8, 12 and 13 and to specify the associations between genetic findings and clinical as well as psychiatric diagnoses.

Patients and methods

Patients

Between 1993 and 1995, 167 consecutive children with enuresis, aged 5–11 y, were examined and treated prospectively at the Department of Child Psychiatry of the University of Cologne, Germany, in a study approved by the local ethics committee. Clinical diagnoses were in accordance with ICD-10 definitions: Functional enuresis was characterized as involuntary voiding of children older than 5 y. Only children with at least one wetting episode per week were included. Exclusion criteria were mental retardation (IQ < 70) and any form of structural or neurogenic form of urinary incontinence. Nocturnal enuresis was divided into three subtypes: primary monosymptomatic nocturnal enuresis, primary non-monosymptomatic nocturnal enuresis (i.e. night wetting with daytime micturition problems) and secondary nocturnal enuresis (dry period of at least 6 months). Daytime incontinence was classified as voiding postponement, urge incontinence or sphincter-detrusor-dyscoordination.

For molecular genetic analyses, 61 children and their families were selected, in mutual consensus conferences with Dr Eiberg, from these 167 patients according to the following criteria: diagnosis of one of the three forms of nocturnal enuresis, families segregating with nocturnal enuresis over two or three generations (i.e. at least one first degree relative affected or carrier of enuresis), core family size at least four members and informed consent from the parents. In 19 patients linkage analyses were not possible because both parents had been enuretic in childhood or siblings were too young for diagnosis. The remaining 42 children, 71.4% (30) boys and 28.6% (12) girls, belonged to 35 different families, as in 7 families two siblings with nocturnal enuresis were included into the study.

Clinical analyses

Clinical diagnoses were based on the following examinations and tests: a careful developmental, paediatric and child psychiatric history including a pedigree, physical and neurological examination, sonography, uroflow with pelvic-floor EMG, bacteriology and urine status, 24-h-micturition-protocol, EEG and a parents’ questionnaire about voiding problems and micturition habits.

Behavioural symptoms were assessed with the Child-Behaviour-Checklist (CBCL), a parental questionnaire. It consists of competence- and problem-scales, including 3 combined scales of internalizing or externalizing behaviour and the total score. The combined scores were evaluated with a cut-off-point at T-score 63 for behavioural problems in clinical range. Psychiatric diagnoses were assigned in consensus conferences according to clinical criteria of ICD-10.

Genetic analyses

Venous blood was collected in EDTA tubes from 42 children and their relatives. The samples were frozen and sent by express courier to the University Institute of Medical Biochemistry and Genetics at Copenhagen, Denmark, for processing and analyses. Lymphocyte DNA was prepared from the venous blood samples by standard methods (13, 14) and used to detect polymorphisms in microsatellite tandem repeats. The microsatellite polymorphisms were analysed by polymerase chain reaction (PCR) and flanking oligonucleotide primers. The five microsatellite markers used for molecular genetic analysis were: D13S291, D13S263, D12S80, D12S43 and D8S260. In the last 6 families D8S257 was used as second marker on chromosome 8q. There were technical problems with marker D8S84, which we had planned to use first for analysis.

Hypotheses and statistics

The following questions were examined: Do the five markers on chromosomes 8q, 12q or 13q identify a common chromosome interval linked to nocturnal enuresis in families segregating with the disorder? Is there a clear association between genetic findings and phenotypes of nocturnal enuresis, mainly between primary monosymptomatic nocturnal enuresis and markers on chromosome 13q? Is there an association between genetic results and somatic as well as psychiatric findings in nocturnal enuresis? Linkage analyses were performed by comparing DNA-polymermorphisms with phenotypes of enuresis in each family. Linkage was classified as possible if affected children inherited the same DNA polymorphisms from their affected parent in contrast to the healthy relatives, and if the lod scores for both markers on a chromosome were positive, or if the lod score for one marker was positive and the other one zero. Families with one positive and one negative lod score for the two markers on chromosome 13q or 12q were described separately and interpreted as a recombination in the analysed interval. Lod scores were calculated by the LINKAGE program. For calculation, a penetrance of 90% according to our formal genetic estimations and a gene frequency of 5% according to the literature (8, 12) were used. Statistics were performed with the SPSS/PC+ program by calculating descriptive variables, cross-tables and performing χ² tests.

Results

Description of patients

The mean age of all 42 children was 8.11 y, with a minimum
of 5.67 y and a maximum of 10.8 y. Thirty-one (73.8%) of the 42 children had nocturnal enuresis: 17 children (40.5%) had primary monosymptomatic nocturnal enuresis, 10 children (23.8%) primary non-monosymptomatic nocturnal enuresis and 4 children (9.5%) secondary nocturnal enuresis. The remaining 11 children suffered from combined primary nocturnal enuresis and daytime wetting, either voiding postponement (8) or urge-incontinence (3).

**Formal genetics**

Blood samples were taken from 172 members of the 35 families. That means, in addition to the 42 patients, 130 additional members from 3 generations were documented and analysed. Seventy family members were parents, 41 siblings, 6 grandparents (families 122 and 124), 4 uncles (families No. 99 and 124), 1 aunt and 8 cousins (family 69). These relatives had following history of enuresis: 12 (28.6%) of the 35 mothers had a history of enuresis, 9 with primary nocturnal enuresis, 2 with secondary nocturnal enuresis and 1 with combined nocturnal and diurnal wetting. One has been suffering form urge symptoms till today, one complains of nocturia. Twelve (29.3%) of the 41 siblings had a diagnosis of enuresis: 9 primary nocturnal enuresis, 2 secondary nocturnal enuresis and 1 nocturnal enuresis plus urge incontinence. If the 7 siblings are added, who were study patients themselves, 19 (39.6%) of 48 siblings were enuretic. In total, 36 (32.4%) of the 111 relatives of the core families (parents and siblings) were affected by enuresis.

Thirty-two (91.4%) families showed an autosomal dominant mode of inheritance of nocturnal enuresis, 24 families with high penetrance and 8 families with lower penetrance. In three families, inheritance could be compatible with an autosomal recessive mode.

**Molecular genetics**

In 23/35 families (65.7%) representing 27/42 children, a positive indication of linkage of nocturnal enuresis to markers on at least one chromosome was found. In 9/27 children linkage to markers on 2 chromosomes was possible, in 2 children even to markers on 3 all chromosomes (families 81 and 168). In 15 children and their 12 families, no linkage to markers on at least one chromosome was possible.

Table 1 shows the lod scores of all 35 families for the five markers on chromosomes 13q, 12q and 8q. In 5 families (No. 39, 46, 47, 75 and 137) the molecular genetic data for chromosome 12q are missing.

In 13 families with 16 children (families No. 47, 50 + 51, 69, 75, 76 + 77, 81, 107 + 108, 120, 126, 149, 152, 162, 168) linkage of nocturnal enuresis to one or both of the markers D13S291 and D13S263 on chromosome 13q was possible. Linkage of nocturnal enuresis to one or both of the markers D12S80 and D12S43 on chromosome 12q was possible in 11 families with 13 children (families No. 62, 69, 71, 76 + 77, 81, 88, 92 + 128, 105, 120, 166, 168). There was an indication of linkage of nocturnal enuresis to marker D8S260 on chromosome 8q in 11 families (families No. 46, 47, 81, 88, 109, 126, 137, 149, 162, 168, 182).

In one family (No. 52) a recombination was found in the interval between the two markers on chromosome 13q, in four families (No. 107, 121, 126 and 182) between the two markers on chromosome 12q.

Phenotypically, linkage to all five markers was possible with all types of wetting. Although nocturnal enuresis was the common diagnosis for all children, two discrete tendencies became apparent: there was a high rate of day wetting problems among children with possible linkage to chromosome 12q and a high rate of primary monosymptomatic nocturnal enuresis among children with possible linkage to markers on chromosome 13q. (Table 2)

Figure 1 shows the pedigrees of 4 families segregating with nocturnal enuresis.

In family 109 the mother and two of the three daughters were affected with primary nocturnal enuresis (index patient monosymptomatic form). DNA polymorphisms of the markers D8S260 and D8S257 segregate with the disorder.

In family 92 the father was affected with primary nocturnal enuresis, son III.2 with primary nocturnal enuresis plus urge incontinence and son III.3 with secondary nocturnal enuresis plus voiding postponement. DNA polymorphisms of the markers D12S80 and D12S43 segregate with the disorder.

In family 149 two of the three sons were affected with primary (son III.2) respectively secondary (III.3) nocturnal enuresis; their younger brother was healthy. The mother suffered from nocturia and was supposed to be a carrier, because her sister and two nephews were enuretic in childhood. DNA polymorphisms of the markers D13S263, D13S291 and D8S260 segregate with the disorder.

In family 135 the mother and the two daughters were affected with primary nocturnal (index patient monosymptomatic form), father and brother were not affected. Linkage to all 5 markers could be excluded. (Figure 1)

**Somatic findings**

Mean residual urine of the 42 children after micturition was 7.37 ml (SD 7.71), mean diameter of bladder wall 2.65 mm (SD 0.62). 22 of the 42 children had a bladder wall thickness greater than 2.5 mm, 8 a residual urine over 5 ml. Mean values of residual urine and bladder wall thickness were not significantly different in those 27 children with positive linkage to one of the markers compared to the entire group of 42 children.

29 (69.0%) of the 42 children had a normal bell shaped uroflow curve, 7 (16.7%) a plateau and 6 (14.3%) a staccato form. No uroflow curve was fractionated. Pelvic-floor-EMG was characterized as completely relaxed in 26 (61.9%), as variably relaxed in 13 (31.0%) and as not
Day and night wetting
1 (6.3%) 7 (53.8%) 2 (18.2%)
Secondary nocturnal enuresis 3 (15.0%) 0 (0%) 2 (18.2%)
Primary nocturnal enuresis 12 (75.0%) 6 (46.2%) 7 (63.6%)

The types of wetting in patients 13 families with 16 patients 11 families with 11 patients 11 families with 11 patients

families (90% penetrance, 5% gene frequency).

The linkage of nocturnal enuresis to one of the markers was

Table 2. Association between molecular genetic results and types of wetting.

Table 1. Two-point lod scores between nocturnal enuresis and markers D13S263 and D13S291 on chromosome 13q, two-point lod scores between nocturnal enuresis and markers D12S80 and D12S43 on chromosome 12q and one-point lod score between nocturnal enuresis and marker D8S260 in 35 families.

Table 2. Association between molecular genetic results and types of wetting.

Frequencies of possible linkage of enuresis to chromosomes

Psychiatric findings

11 (26.2%) of the 42 children had at least one psychiatric diagnosis, 2 (4.8%) an emotional, 7 (16.7%) an expansive disorder and 5 (11.9%) had encopresis. Out of the 27 children with possible linkage to one of the

<table>
<thead>
<tr>
<th>Types of wetting in patients</th>
<th>13q</th>
<th>12q</th>
<th>8q</th>
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<td>13 families with 16 patients</td>
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<tr>
<td>Primary nocturnal enuresis</td>
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<td>6 (46.2%)</td>
<td>7 (63.6%)</td>
</tr>
<tr>
<td>Monosymptomatic PNE</td>
<td>8 (50.0%)</td>
<td>3 (23.1%)</td>
<td>4 (36.4%)</td>
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<tr>
<td>Non-monosymptomatic PNE</td>
<td>4 (22.2%)</td>
<td>3 (23.1%)</td>
<td>3 (27.3%)</td>
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<tr>
<td>Secondary nocturnal enuresis</td>
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*Marker and ^recombination fraction.

relaxed in 3 (7.1%) of the 42 children. The distribution of uroflow patterns among the 27 children with possible linkage of nocturnal enuresis to one of the markers was comparable, only the rate of 70.4% (19) completely relaxed pelvic floor EMGs was slightly higher than in the whole group.
analysed markers 9 (33.3%) had at least one psychiatric diagnosis. According to parental assessment in the CBCL, 9 (21.4%) children had behavioural symptoms in the clinical range (total), 3 (7.1%) clinical internalizing and 8 (19.0%) externalizing behavioural scores. The CBCL behavioural scores were not different among the 27 patients with possible assignment of nocturnal enuresis to a marker than among the entire group.

Discussion

This study represents the largest group of children with nocturnal enuresis studied with both formal and molecular genetics, so far. In contrast to previous studies, all 5 microsatellite markers with a positive linkage to nocturnal enuresis were tested on the same patients. In addition, the children were examined prospectively with a broad, standardized battery of non-invasive methods and tests, so that a detailed description of the somatic and behavioural phenotype was possible.

Formal genetic analyses of the 42 children eligible for further evaluation show a high rate of first degree relatives being affected with enuresis. As a selected group, families with both parents being enuretic, were ruled out. The rate therefore is lower than in Bakwin's formal genetic analyses (5,6). In addition to sporadic cases, the pedigrees are compatible with autosomal dominant and rarely, with autosomal recessive modes of inheritance (12).

The present molecular genetic results also demonstrate locus heterogeneity in nocturnal enuresis. In families with several affected members, linkage of nocturnal enuresis to at least three different gene loci on chromosomes 13q, 12q and 8q was possible. The exact number of families with possible linkage to each of the chromosome intervals, however, should be interpreted carefully, as in small families the possibility that a specific chromosome interval segregates by chance must be taken into consideration.

It is evident that the 5 markers do not identify a common chromosome interval linked to nocturnal enuresis in all families, not even in those families with primary monosymptomatic nocturnal enuresis. In one family, linkage to all 5 markers could be excluded, which implies that other, previously not identified markers could be involved with an even greater degree of genetic heterogeneity.

There is also clear heterogeneity of the clinical phenotype. Most importantly, children of families with possible linkage to one of the markers were not restricted to primary monosymptomatic enuresis, as previously described by Eiberg (8), but included children with primary non-monosymptomatic nocturnal enuresis, secondary nocturnal enuresis and combined day and night wetters. Similarly, among the affected relatives all types of wetting occurred.

These findings are in congruence with epidemiological data: Thus, Fergusson et al hypothesized that primary and secondary nocturnal enuresis are not distinct disorders, but share a common biological basis leading to a late age of attaining dryness in primary nocturnal enuretics and a vulnerability towards stressful life events in secondary enuretics (15). Although interpretation is limited by the small family-sizes, there were, however, slight trends towards an association between possible linkage to chromosome 13q and primary monosymptomatic nocturnal enuresis as well as between combined night and day wetting and possible linkage to chromosome 12q.

The somatic findings were also highly variable. The fact that 11 of the examined 42 children were combined day and night wetters might explain the high rate of pathological sonographic, uroflowmetric and pelvic-floor-EMG findings. There was no trend for somatic findings according to possible linkage to a certain chromosome.

Psychiatrically, both the group of 42 children analysed, as well as the 27 children with possible linkage were characterized by an exceptionally low rate of behavioural problems: only one fifth to one quarter of children fulfilled the criteria of at least one psychiatric diagnosis according to ICD-10. If children showed problems, these were almost always expansive diagnoses like hyperkinetic syndrome and conduct disorder. This incidence rate is low compared to the whole study population of 167 children with all forms of enuresis (40.1% at least one psychiatric diagnosis) and in the same range as the incidence of psychiatric disturbances among patients with primary nocturnal enuresis (19.5% at least one psychiatric diagnosis) (16, 17).

Enuresis is characterized by a complex interaction of psychiatric and somatic factors, that result from genetic constitution under influence of environment. So far, no candidate gene could be identified. Based on the variations of circadian rhythm of AVP in nocturnal enuresis (4), the arginine vasopressin gene was initially discussed as a possible candidate gene for nocturnal enuresis. This could be definitely excluded as well as two neuroreceptor genes on chromosome 13q, HTR2 (serotonin 5-HT-2 receptor) and EDNRB (endothelin receptor) (8) and a variety of candidate genes, including the glucosamine-6-sulphatase, high mobility group proteins (HMG1), gamma interferon and MDM2 genes. The aquaporin-2 (AQP2) gene needs a more precise localization before it can be considered as a candidate (12).

In summary, the present findings of possible linkage to three different chromosome intervals support the hypothesis of genetic heterogeneity of nocturnal enuresis described in previous molecular genetic studies of Eiberg (9, 10) and Arnell (11, 12).

In addition, the analyses show that a common major gene for nocturnal enuresis does not exist on any of the analysed chromosome intervals, not even for primary monosymptomatic nocturnal enuresis. There were no clear associations between genetic findings and phenotypes of nocturnal enuresis. Phenotypically, the forms of wetting of children with possible linkage were heterogeneous and not restricted to primary monosymptomatic nocturnal enuresis. No association between genetic results and somatic or psychiatric findings was found. An exceptionally low rate
Nocturnal enuresis and molecular genetic analyses

Fig. 1. Pedigrees of four families segregating with nocturnal enuresis: in family 92 linkage to two markers on chromosome 12q, in family 109 to two markers on chromosome 8q and in family 149 linkage to the markers on two chromosomes (13q and 8q), was possible. The affected children inherited the same DNA polymorphisms from their affected parent in contrast to their healthy siblings. In family 135, linkage to markers on all three chromosomes could be excluded.
of behavioural problems was apparent in this group of patients.

As the power of linkage analyses depends on a specific classification of the phenotype, detailed clinical investigations of the affected individuals are of utmost importance. Therefore further clinical and molecular genetic research, especially in large multigenerational families is needed.

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