

# Minor disease features in neurofibromatosis type 1 (NF1) and their possible value in diagnosis of NF1 in children $\leq 6$ years and clinically suspected of having NF1

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## Abstract

**Objective**—To establish the frequency of minor disease features in children with neurofibromatosis type 1 (NF1) and to evaluate the value of minor disease features in children  $\leq 6$  years with a suspected diagnosis of NF1, considering that the disease is virtually 100% penetrant at 6 years of age.

**Design**—During this 10 year, prospective, multidisciplinary, follow up study, 209 children suspected of having NF1 were examined; 150 were diagnosed with NF1 and 59 were not. The present analysis included children in whom NF1 was considered to be present at 6 years of age ( $n=85$ ) and children without NF1 at 6 years of age ( $n=42$ ).

**Results**—The minor disease features macrocephaly (52.9%), short stature (24.7%), hypertelorism (63.5%), and thorax abnormalities (37.6%) were highly prevalent in children with NF1 and significantly associated with a diagnosis of NF1 at 6 years of age. In addition, the mean number of minor disease features was significantly higher in children with NF1 at 6 years of age compared to the group without a diagnosis at 6 years of age (mean 1.8 v 0.8,  $p<0.001$ ). Moreover, children with three or more minor disease features were all diagnosed with NF1 under the age of 6 years. Multivariate analysis using a logistic regression model showed that macrocephaly, short stature, hypertelorism, and thorax abnormalities were all independently associated with the presence of NF1 at 6 years of age.

**Conclusion**—In children with insufficient diagnostic criteria aged  $\leq 6$  years, documentation of minor disease features may be a helpful aid in predicting the diagnosis of NF1 in years to come.

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**Keywords:** neurofibromatosis type 1; minor disease features; macrocephaly; short stature

Type 1 neurofibromatosis, NF1, is a common genetic disorder with autosomal dominant inheritance. Half of all patients represent sporadic cases. Diagnostic criteria were established by the National Institutes of Health

(NIH) and two or more criteria must be present for the diagnosis. In cases where a first degree family member is affected, only one additional criterion is sufficient for a diagnosis.<sup>1</sup> The penetrance of the disease is regarded as virtually 100% at 5 years of age<sup>2</sup> and the diagnostic criteria have been shown to be applicable in children from 6 years of age.<sup>3</sup>

Although the NF1 gene was cloned in 1990,<sup>4-6</sup> mutations in this large and complex gene are detected in only 10-20% of NF1 patients using classical methods such as polymorphic markers, single strand conformational analysis (SSCP), and Southern analysis on DNA from lymphocytes.<sup>7</sup> Accordingly, mutation analysis is not available as a diagnostic tool and in the majority of NF1 patients a diagnosis will still be based on the presence of two or more clinical criteria.

In addition to the diagnostic signs, physical characteristics such as macrocephaly, short stature, hypertelorism, and thorax abnormalities are observed more frequently in NF1 patients than in the general population. As such, these characteristics are considered minor disease features.<sup>8</sup>

In this study, we aimed to assess the frequency of minor disease features in children with NF1 and to evaluate the association of the presence of minor disease features with a diagnosis of NF1 in children at 6 years of age. We hypothesised that in contrast to the diagnostic criteria which develop with age, macrocephaly, short stature, hypertelorism, and thorax abnormalities are present from birth and therefore may be of value in predicting NF1. In this way, parents of children suspected of NF1 and younger than 6 years of age can be informed of the necessity for diagnostic follow up and both parents and children may benefit from the knowledge that many of the child's symptoms are the result of an underlying disorder.

## Patients and methods

### STUDY POPULATION

Between July 1985 and January 1996, a multidisciplinary NF1 team in the Sophia Children's University Hospital in Rotterdam, including a paediatrician, dermatologist, paediatric neurologist, ophthalmologist, and clinical geneticist, evaluated children suspected of having NF1. Children were referred with a suspected diagnosis of NF1 by general practitioners and medical specialists. Age at referral was variable

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Table 1 Age distribution at diagnosis within groups with NF1 (n=150) and without NF1 (n=59)

|          | NF+ (n=150)  | NF- (n=59)   |
|----------|--------------|--------------|
| ≤6 years | 85 (group 1) | 17           |
| >6 years | 65           | 42 (group 2) |

Patients in groups 1 and 2 were selected for this analysis.

(range 1.2-14.5 years, mean age at first examination 5.3 years). Reasons for referral were suspected symptoms of NF1, a positive family history of NF1, or insufficient diagnostic criteria and minor disease features. Diagnosis was based on the NIH criteria, assuming full penetrance of NF1 at 6 years.

Follow up examinations were performed regularly according to a standardised protocol. Initial assessment included the patient's history (for example, age, sex, type of schooling, and presence of additional support by speech therapist or physical therapist), physical examination (length, weight, head circumference, presence of dysmorphic features), and motor coordination tests supplemented by additional diagnostic tests if required.<sup>9</sup>

MINOR DISEASE FEATURES

The following physical features were defined as minor disease features and can be considered to be present from birth. Macrocephaly is a head circumference equal to or exceeding the 97th centile (specified for age and sex), irrespective of other body measurements and head circumference of family members. Children diagnosed with hydrocephalus were excluded from this group (n=1). Short stature is defined as a height equal to or below the 3rd centile (specified for age and sex), irrespective of other body measurements and height of family members. Children with NF1 and endocrinological disorders leading to short stature were excluded (n=6). Hypertelorism was recorded at clinical assessment by an experienced paediatrician (clinical impression of hypertelorism). Inter- and outer canthal distances were not measured. Thorax abnormalities scored included both pectus excavatum and pectus carinatum.

Other minor disease features such as learning difficulties, speech abnormalities, and behavioural and motor problems were not analysed because of lack of objective standards.

ANALYSES

In order to assess the frequency of minor disease features in children with NF1 and to evaluate the association of the presence of

minor disease features with the presence or absence of NF1 in children at 6 years of age, two groups were selected from the total population. The first group (group 1) consisted of children diagnosed with NF1 at or before the age of 6 years. The second group (group 2) included children without a diagnosis of NF1 at 6 years of age. All children were initially suspected of having the disease when they were younger than 6 years of age.

All data were registered on a standardised form and analysed using SPSS 6.0. In univariate analyses, associations between the presence or absence of minor disease features and NF1 diagnosis were assessed using Fisher's exact test. Two sided p values were calculated at a significance level of 0.05. The number of minor disease features was computed in both patient groups and a difference in the mean number was tested for significance using the t test for independent samples.

In addition, multivariate analyses were performed in order to assess the independent value of each minor disease feature in the diagnosis of NF1, using a logistic regression model. Variables significant in the univariate analysis (p<0.05) were included in the logistic regression model. Variables were excluded from the multivariate model on the basis of the likelihood ratio test if the p value was lower than <0.05.

Results

PATIENTS

During this prospective 10 year follow up study, 209 children suspected of having NF1 were examined; 150 were eventually diagnosed as having NF1 and 59 were not (prevalence of NF1 in children referred to the clinic 71.8%). In 85 (49 boys, 36 girls) of the 150 children with NF1, the disease was diagnosed at or before 6 years of age (group 1). The age at diagnosis of the remaining 65 children was >6 years owing to diagnostic delay.<sup>10</sup> Forty-two (26 boys, 16 girls) of the 59 children did not have NF1 at 6 years of age (group 2) (table 1) and were therefore >6 years at last examination. The remaining 17 children without NF1 were younger than 6 years of age and could therefore still develop NF1. Hence, they were excluded from the present analysis as we focused on children without a diagnosis of NF1. Age at diagnosis was dependent on age at presentation of symptoms and on age at referral. Children without a diagnosis of NF1 at the end of follow up appeared to have been initially referred to the clinic on the basis of a positive family history of NF1 or had insufficient diagnostic criteria and minor disease features. In 14 of these 42 children (33.3%), one diagnostic criterion was present other than an affected first degree relative. In 28 of these 42 children (66.7%) only a positive family history was present.

UNIVARIATE ANALYSIS

Table 2 presents the frequencies of minor disease features in patients with and without NF1 at 6 years of age. Macrocephaly was present in 52.9% of children with NF1, short

Table 2 Associations between minor disease features in children with and without NF1 at 6 years of age

| Characteristics                   | Group 1<br>NF1+ (n=85) | Group 2<br>NF1- (n=42) | OR; 95% CI     | p value |
|-----------------------------------|------------------------|------------------------|----------------|---------|
| Sex (% males)                     | 42.4                   | 38.1                   | (-0.22; 0.143) | 0.093   |
| Positive family history (%)       | 52.9                   | 52.4                   | (-0.19; 0.18)  | 0.953   |
| Mean age at first examination (y) | 3.9                    | 7.7                    | (-5.25; -2.36) | <0.001* |
| Mean age at last examination (y)  | 8.3                    | 10.8                   | (-3.99; -0.97) | 0.001*  |
| Mean follow up time (y)           | 4.4                    | 3.1                    | (-0.11; 2.79)  | 0.070   |
| Macrocephaly (%)                  | 52.9                   | 31.0                   | (-0.40; -0.04) | 0.017*  |
| Short stature (%)                 | 24.7                   | 4.8                    | (-0.31; -0.85) | 0.001*  |
| Hypertelorism (%)                 | 63.5                   | 31.0                   | (-0.50; -0.15) | <0.001* |
| Thorax abnormality (%)            | 37.6                   | 9.5                    | (-0.42; -0.14) | <0.001* |

CI=confidence interval; \*significant at level p<0.05.

Table 3 Number of minor disease features (%) in children with and without NF1 at 6 years of age

| Number of minor disease features | Group 1 NF+<br>n=85 (%) | Group 2 NF1-<br>n=42 (%) |
|----------------------------------|-------------------------|--------------------------|
| 0                                | 11 (12.9)               | 18 (42.8)                |
| 1                                | 17 (20.0)               | 16 (38.1)                |
| 2                                | 38 (44.7)               | 8 (19.1)                 |
| 3                                | 17 (20.0)               | —                        |
| 4                                | 2 (2.4)                 | —                        |

Median test; p value <0.001.

stature in 24.7%, hypertelorism in 63.5%, and thorax abnormalities in 37.6%. Comparison of the frequencies of minor disease features between both patient groups showed that macrocephaly, short stature, hypertelorism, and thorax abnormalities were significantly associated with the presence of NF1 at 6 years of age (table 2).

Hypothetically, if the associations between minor disease features and a diagnosis of NF1 at 6 years of age were the result of an excess of minor disease features in children with NF1 and aged  $\leq 3$  years at the time of diagnosis, the additional value of minor disease features in diagnosing children  $\leq 6$  years of age would be minimal. Therefore, subgroup analyses were performed excluding children diagnosed at  $\leq 3$  years of age. Although sample numbers decreased, the results were similar. The association between minor disease features and the presence of NF1 was still significant (data not shown).

The number of children with multiple minor disease features was significantly higher in group 1 than in group 2. The mean number of minor disease features was 1.8 and 0.8 respectively (table 3). Children with three or more minor disease features were all diagnosed with NF1 under the age of 6 years. The cumulative percentages of children with two or more minor disease features were also significantly higher among patients in group 1 (67.1%) compared to group 2 (19.1%). In addition, 12.9% of children had no minor disease features in group 1, compared to 42.8% of children in group 2. This difference was also highly significant (median test,  $p < 0.001$ ).

#### MULTIVARIATE ANALYSIS

Table 4 shows the results of the multivariate logistic regression analysis. Each minor disease feature was independently associated with the presence of NF1 at 6 years of age. Odds ratios varied from 2.1 for macrocephaly to 8.0 for short stature. Short stature (OR=8.0, 95% CI=6.45-9.59) and thorax abnormalities (OR=5.7, 95% CI=4.42-6.90) were the most important independent predictors of the presence of NF1 at 6 years of age.

Table 4 Relative risk (95% CI) according to presence or absence of minor disease features for the eventual diagnosis of NF1 in children  $\leq 6$  years of age at presentation

| Variables          | $\beta$ | OR  | 95% CI      |
|--------------------|---------|-----|-------------|
| Constant           | -10.6   |     |             |
| Macrocephaly       | 0.73    | 2.1 | (1.19-2.97) |
| Short stature      | 2.08    | 8.0 | (6.45-9.59) |
| Hypertelorism      | 1.15    | 3.1 | (2.26-4.03) |
| Thorax abnormality | 1.73    | 5.7 | (4.42-6.90) |

$\beta$ =coefficient; OR=odds ratio; CI=confidence interval.

For the same reasons as mentioned above, we constructed a logistic regression model excluding children diagnosed  $\leq 3$  years of age. Odds ratios were similar to those computed in the overall analysis, although confidence intervals were wider, but did not include OR=1.

#### Discussion

The minor disease features macrocephaly, short stature, hypertelorism, and thorax abnormalities are very common in children with NF1, ranging from 24.7% for short stature to 63.5% for hypertelorism. A significant association was found between their presence and a diagnosis of NF1 at 6 years of age. Furthermore, the mean number of minor disease features in children diagnosed with NF1 at 6 years of age is significantly higher than in children without NF1 at 6 years of age. Multivariate analyses showed odds ratios varying from 2.1 for macrocephaly to 8.0 for short stature, confirming an independent association between each variable and NF1 diagnosis at 6 years of age. The highest odds ratios were observed for short stature (8.0) and thorax abnormalities (5.7).

Our findings suggest that, in the practice of a multidisciplinary clinic, the probability of developing NF1 in the presence of insufficient diagnostic criteria may be predicted on the basis of minor disease features. We have attempted to quantify this probability in order to define a group of children in which follow up is essential as the risk of developing NF1 is substantial. In this way, parents of children suspected of having NF1 and younger than 6 years of age can be informed of the necessity for diagnostic follow up and both parents and children may benefit from the knowledge that many of the child's symptoms are the result of an underlying disorder.

Minor disease features could be of special importance to children with a de novo mutation in the NF1 gene as they may be diagnosed at a later age than children with an affected relative. The importance and applicability of the diagnostic criteria in children is generally accepted. Korf *et al*<sup>11</sup> showed that 24 of 41 (58%) children went on to develop NF1, according to NIH criteria, after an initial visit during which only café au lait spots were observed. Almost all children in this study were diagnosed within three years of follow up and before 5 years of age. Obring *et al*<sup>8</sup> classified 151 out of 160 children (94%) under 6 years of age on the basis of the diagnostic criteria, including 80% of children with a negative family history. We emphasise that minor disease features should only be used to predict the likelihood of NF1 diagnosis when children have been checked thoroughly for the presence of conventional diagnostic criteria by NF1 specialists.

In the Welsh study, similar prevalences were reported of macrocephaly (45%) and short stature (31.5%).<sup>12</sup> Riccardi<sup>13</sup> also reported short stature (more than one-third below the 5th centile<sup>14</sup>) and pectus excavatum (31%) to be present in a high percentage of his patients. As such, the association between minor disease

features and NF1 is not surprising. However, the possible value of these variables in diagnosing NF1 has so far not been discussed.

We realise that the group of children without NF1 at 6 years of age may still contain children with an NF1 gene mutation. Particularly, children with one diagnostic criterion other than a positive family history in combination with several minor disease features remain suspect. Atypical NF1 patients have been described with reduced penetrance<sup>15</sup> or late expression of the disease.<sup>13</sup> In addition, cases of germline and somatic mosaicism have been reported.<sup>16-18</sup> Follow up of the children not diagnosed with NF1 at 6 years of age may result in additional diagnoses. Nevertheless, associations between minor disease features and a diagnosis of NF1 at 6 years of age would not be influenced by these cases, as omitting them would only make the association stronger.

The data on hypertelorism are crude as we did not use physical measurements. Therefore we recommend validation of the associations and the logistic model in a similar setting in which hypertelorism is measured. Furthermore, although children were referred suspected of NF1, it is possible that they were referred on the basis of insufficient diagnostic criteria and minor disease features, leading to an underestimation of the odds ratios. In contrast, an overestimation may have been made owing to referral at a later age, with insufficient diagnostic criteria and fewer minor disease features.

Easton *et al*<sup>19</sup> presented evidence that modifying genes may play a major role in the variability of NF1 within families. Presumably, modifying genes may also be of influence on the presence of minor disease features in NF1 patients. In time, minor disease features may be of importance in discovering loci for these modifying genes.

As mutation analysis becomes more important in diagnosing (atypical) NF1 patients, minor disease features may also gain importance in isolating those (atypical) patients suspected of an NF1 gene mutation. It remains difficult to perceive that a positive family history in a child with a reduced expression of the disease and aged >6 years will lead to a diagnosis and the same expression in a child (>6 years) without affected family members will not. In the future, it may clarify matters to exclude a positive family history from the diagnostic criteria for NF1. Basically, disease mechanisms are dependent on the gene mutation, synthesis of mRNA and protein, the genetic background, and environmental factors and independent of a positive family history.

Diagnoses other than NF1 should always be considered in children with insufficient diagnostic criteria for NF1. In our study one of the

14 children >6 years, with only one (clinical) diagnostic criterion, was diagnosed as having neurofibromatosis type 2 during follow up (dermal neurofibromas, bilateral vestibular schwannomas), one child was diagnosed as having Leopard syndrome, and a third child was diagnosed as having Proteus syndrome. No minor disease features were present in these three children. No alternative diagnoses could be made in the remaining children. Other studies include Bannayan-Riley-Ruvalcaba syndrome, McCune-Albright syndrome, urticaria pigmentosa, multiple lipomas, congenital generalised fibromatosis, and steatocystoma multiplex in the differential diagnosis.<sup>8, 13</sup>

In conclusion, in children with insufficient diagnostic criteria, under the age of 6 years, and examined in a multidisciplinary NF1 clinic, documentation of minor disease features may be a helpful aid in diagnosing NF1.

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