Genetic and clinical aspects of neurofibromatosis type 1: study of a large sample of affected brazilian individuals

INTRODUCTION

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen’s disease, is a very common autosomal dominant condition, with a prevalence from birth to adolescence of about 1/3,000 in most human populations. NF1 affects the growth of neural tissue cells, thus determining the development, on different locations, of benign tumours that emerge in any life stage. Its manifestations are usually progressive and eventually result in a significant increase of both mortality and morbidity rates among older patients.

The heterozygous genotype of NF1 has an almost complete penetrance rate and exhibits an extremely variable expressivity. Its gene possess clear pleiotropic manifestations. Like most autosomal dominant conditions, a relatively large rate of cases of NF1 (50-60%) are due to de novo mutations. The adaptive (fitness) value of the affected individuals was estimated in rates that range from 50% to 80%. Classically, NF1 is classified among a relatively heterogeneous group of diseases known in the medical literature as phacomatoses, conditions characterized by the presence of hamartomas, pre-tumoral tissue disorganization of embryonic origin. Four characteristics are important for the diagnosis of NF1: café-au-lait spots, ephelides (freckles), skin neurofibromas, and Lisch nodules. Café-au-lait spots are generally present in numbers (six or more) and dimensions (diameter larger than 0.5 cm in children or 1.5 cm in adults) only exceptionally found among not-affected individuals. Ephelides are small (1-3 mm) hyperpigmented skin spots of intertriginous nature, situated preferentially on skin folds (axillary, inframammary, inguinal, cervical, and upper palpebral regions). Neurofibromas arise primarily from growth regulation disturbances that take place in Schwann cells, expressing themselves as tumors of smooth texture that include Schwann and perineural cells, fibroblasts, axons, mast cells, and elements from the extra-cellular matrix. More complex and severe forms are plexiform neurofibromas, that contain also non-neural surrounding tissue elements (connective, muscular, and vascular). The Lisch nodules of the irides, which are believed to be pathogenic of the NF1, are small spherical brownish hamartomas easily detected at examination with the slit lamp. Other manifestations, less frequently observed but however important for the diagnosis of the condition, include a series of general, neurological, and skeletal secondary manifestations that arise due to compression,...
erosion, or malignancy transformation. Two of these secondary manifestations, bone defects and optic pathway gliomas, together with the four main ones previously cited, make up the cardinal signs of relevance for the clinical diagnosis of NF1, as established by a National Institutes of Health consortium (1).

We were able to collect, in the present report, a large set of families with cases of NF1. As far as we know, this sample of personally examined NF1 patients is the largest thus far reported in the literature. In spite of most formal phenotypic and genetic aspects of NF1 having already been explored and known in detail, the size of our sample enabled us to describe in detail the occurrence and body distribution of skin and pigmentation defects and to estimate the frequencies of all cardinal signs and of six secondary signs and symptoms; these were compared to the corresponding frequencies among non-affected individuals and will be described in detail in another publication.

MATERIAL AND METHODS

In the present paper we describe the results of our studies, performed in a sample of 209 Brazilian patients affected by neurofibromatosis type 1 (von Recklinghausen's disease), examined in a project developed from 1997 to 2006. This group included reexamined NF1 patients from five families seen in another similar project developed in 1988-89. Two of our 209 patients had been adopted and no family history was available. This explains the fact that some parameters were estimated with a sample size of 207 affected individuals instead of 209 and a total number of families of 117 instead of 119. Patients or their guardians provided written informed consent and the study was approved by the National (Brazilian) Research Ethics Committee (CONEP).

The phenotypic description of our sample included the determination of average values and frequency estimates of 25 measurements, signs and symptoms, according to their occurrence (isolated or familial cases), and to the sex and age of affected individuals.

Weight, height, and occipito-facial (head circumference or OFC) measurements as well as ocular and whole body photographs were taken from most adult patients. Skin pigmentation defects such as café-au-lait spots and ephelides, simple and plexiform neurofibromas, and other lesions occasionally present, were photographed, counted, measured, and mapped into standard regions of skin radicular innervation (Diem and Lentner, 1968); the innervation delimitation lines were later removed from the final dia-
GRAMS we prepared (example shown in Figure 1A). Eye photographs for recording the presence of Lisch nodules (Figure 1B) and other ocular alterations were obtained with an AF micro Nikkor 105mm objective attached to a reflex Nikkon N70 professional camera. General procedures of physical examination were applied in all patients for assessing the presence of minor cranio-facial dysmorphisms, body asymmetries, skeletal defects, and hernias. All patients were inquired about the occurrence of the following symptoms: dysacusia, cephalae, parestesias, and abdominal pains. In order to assess the relative importance of secondary signs and symptoms commonly investigated in patients affected by NF1 (cephalae, dysacusia, parestesias, white and red spots, abnormal spine curvatures, and backbone pains), these were self-investigated in a random sample – collected among university staff or students and their family members – of 283 normal individuals (258 adults and 25 children), who had been duly instructed to perform a standardized self-examination. They were also asked to record the number, size, and localization of café-au-lait spots and ephelides occasionally presented by them.

Using anamnestic and genealogical data collected from the families with affected individuals, we estimated the values of the following genetic parameters in NF1: proportion of familial and isolated cases, sex-ratio, segregation rate in the offspring of affected individuals, penetrance rate, and the fitness value. The assessment of penetrance and fitness values and the determination of scores used in discriminant and multivariate analysis were based on standard statistical methodology(2,4). Other general statistical methods are found in standard textbooks like Zar's(6). The parental age effect in NF1 was studied by comparing the values found in the group of isolated cases (originated by de novo mutation) to the values estimated among parents of familial cases through nonparametric methods. The parental age effect was also investigated indirectly by applying the exact test for birth order developed by Haldane and Smith(7).

Peripheral blood samples were obtained from the patients and their available first-degree relatives for molecular studies. Tests with DNA polymorphic markers were performed in affected individuals and in their first-degree relatives from all families examined during the period 1997-2001; the results obtained with these and other molecular techniques, however, will be presented elsewhere.

RESULTS AND DISCUSSION

Many aspects of both formal clinics and genetics of NF1 presented here have already been the subject of a relatively large number of publications. The corresponding updated literature revision and discussion will be omitted here but can be found in some comprehensive publications listed in the references section of this paper(8-12).

As indicated before, two of our patients had been adopted and no familial history was available. Among 117 of our 119 families with cases of NF1, the propositi were isolated in 87 and familial in 30. The frequencies of isolated and familial cases among our affected propositi were respectively 87/117 = 0.744 and 30/117 = 0.156. Among all affected cases, 87/207 = 0.420 were isolated and 120/207 = 0.580 familial.

Among all (209) affected individuals, the average age (mean ± 1 s.e.) at examination was 22.88 ± 1.35 years; the corresponding values for isolated and familial cases were respectively 13.63 ± 1.41 and 29.73 ± 1.90 years.

Among our patients, 105/209 = 0.502 were males and 104/209 = 0.498 females. These estimates don’t differ significantly between them as well as from the expected sex ratio 1:1.

The segregation ratios (affected: normal) of the defect in the offspring of affected males vs. normal females (44 couples), normal males vs. affected females (41 couples), and of all affected vs. normal parents (85 couples) were respectively 72:79, 93:69, and 165:148. The analysis of these data showed that the segregation rate doesn’t differ from 1:1 in the three groups (heterogeneity chi-squared value = 2.958; 1 d.f.; P > 0.05). These results are an indirect evidence that the gene penetrance should not differ significantly from unity. More appropriate methods of estimation of the penetrance value take into account not only this segregation ratio but also the observed numbers of affected individuals, obligate normal carriers of the gene and the structure of trees of normal individuals descending from known heterozygotes. In our set of genealogies we located just one single instance of a triplet affected grandfather-normal father-affected offspring. Since the grandfather had been referred to as carrier of only café-au-lait spots, we classified the case occurring in this family conservatively as isolated. Under this assumption, in all families investigated by us we recorded 87 trios of affected individuals in three consecutive generations. The estimate of the non-penetrance value is therefore 0/87 = 0, with a 95% exact confidence interval (13) is 0 to 0.0415; under the hypothesis that the case classified as isolated were actually a familial one, the corresponding non-penetrance value would be 1/88 = 0.011, with a 95% c.i. of 0.03 to 0.0617. Taking all these facts into account, it comes out that the penetrance value of the NF1 phenotype is a figure inside the interval 0.94 to 1 with a probability of about 95%.

Table 1 lists the observed frequencies of the cardinal (primary) and secondary signs and symptoms investigated on a routine basis among our NF1 patients, in the total sample and grouped by type of occurrence (isolated or familial), by sex, and by age (smaller or larger than 15 years). The table shows also the results of chi-squared tests obtained by contrasting these frequencies within each of the three subgroups; significant test probability values are indicated in bold face. Since the table has 25 entries for each subgroup, 75 chi-squared tests were performed and the usual critical level of hypothesis rejection (0.05) was therefore lowered to 0.05/75 = 0.0007 to take this into account (Bonferroni’s correction). While no statistically different frequencies were detected when familial and isolated cases were compared, the symptom cephalaea was markedly more frequent in females than
Table 1 - Primary and secondary signs and symptoms investigated on a routine basis in the present sample of NF1 patients: frequencies observed in the total sample and in subgroups of patients agglutinated by type of familial occurrence [recurring (familial) or isolated], by sex, and by age. Bold face figures indicate statistically significant test values ($P < 0.05/75 = 0.0007$) resulting from comparisons within subgroups.

| Table 2 lists the observed frequencies of primary and secondary signs and symptoms in a sample of 283 non-affected individuals (controls drawn from the general population), compared with the observed ones in our sample of patients. The last column of this table shows the probabilities favoring the diagnosis of NF1, conditional to the presence of the corresponding sign or symptom in a given suspected adult subject [P(NF1|sign)]. The conditional probabilities in favor of neurofibromatosis generated by the presence of the signs and secondary symptoms listed on the table (cephalaea, dysacusa, abnormal spine curvatures, parestesias, cutisspot, and hemangiomas) ranged from 65% to 82%; a suspected adult patient presenting all the six secondary signs/ symptoms has a final probability favoring the diagnosis of NF1 of more than 99%, as we show in a numerical example detailed below.

We summarize in Table 3 the necessary elements for performing the categorical discriminant analysis based on discrete data (frequencies of secondary signs and symptoms among affected and normal subjects). The following abbreviations have been used: Sign/sympt: sign

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This table allows the straightforward determination of conditional probabilities favoring the diagnosis of neurofibromatosis as function of any combination of signs and symptoms present in a suspected subject. For example, in the hypothetical case of a suspected subject presenting all the signs and symptoms listed on Table 3, adding all the logarithms of the column loga+, we get 5.342; applying this figure in $P(\text{NF1}|\text{signs}) = \frac{e^{5.342}}{1 + e^{5.342}}$ we obtain the value of 0.996 favoring the hypothesis of NF1. This last figure and its complementary value $P(\text{normal}|\text{signs}) = 1 - P(\text{NF1}|\text{signs}) = 0.004$ can be combined with the prior probabilities of NF1 (1/3000) and normality (2999/3000), to give the final probability figure favoring the diagnosis of NF1. Thus, if a person is randomly taken from the general population and presents all the signs and symptoms listed on Table 3, the the final probability favoring the hypothesis of NF1 is therefore $P(\text{nf}) = \frac{996}{996 + 11996} = 0.077$, that is about 8%. In the case the suspected subject had been sent by a doctor that performs the right clinical diagnosis in about half of the cases, the final probability coincides with the conditional probability $P(\text{NF1}|\text{signs}) = \frac{e^{5.342}}{1 + e^{5.342}} = 0.996$ derived before.

For the description of the topographical distribution of the café-au-lait spots (cals), we considered as such only spots with a diameter of at least 0.5 cm, grouped by the following skin areas: A) trunk (including the head and, in the case of male individuals, the genital area) and limbs; B) right and left body half; C) anterior and posterior body side. While anterior/poste-
rior and right/left body surface partitions represent identical skin areas, the area partition between trunk and limb surfaces is unequal, since the limb area corresponds to about 55% of an adult body. The numbers of cals have been also calculated for each one out of the eight possible factorial combinations between A, B, and C. The overall average number of café-au-lait spots (mean ± s.e.) was 26.12 ± 1.28. The original data were transformed after the usual square root transformation for counts and analyzed through a standard three-factor model of analysis of variance with replications. The results of this analysis showed that the cals occur preferentially in the trunk (A) of NF1 patients (EMS = 0.51503; AMS = 60.80432; F = AMS/EMS = 118.06; d.f. = 1, 944; P < 0.00001), mainly in its posterior face (C): ACMS = 4.46503; F = ACMS/EMS = 8.67; d.f. = 1, 944; P = 0.004. The average values for the number of cals on trunk and on its posterior face were respectively 16.85 ± 0.85 and 9.32 ± 0.53.

No statistically significant differences were detected when the number of cals were compared between male and female affected individuals.

For the description of the topographical distribution of the neurofibromas, we observed the same criteria used for the study of café-au-lait spots. The overall average number of neurofibromas (mean ± s.e.) was 10.72 ± 2.03 among 74 adult patients.

The data on neurofibroma counts were submitted to the same model of analysis of variance used in the case of café-au-lait spots. The analysis showed that neurofibromas occur much more frequently on the trunk (A) than on the limbs (EMS = 0.55996; AMS = 7.65077; F = AMS/EMS = 13.66; d.f. = 1, 584; P = 0.0005). The average number of neurofibromas on trunk was (mean ± s.e.) 7.09 ± 1.41.

In order to verify the existence of an association between parental age and the incidence of isolated cases of NF1, a finding already established in the specialized literature, we compared the parental ages at birth of affected isolated cases (average values for paternal and maternal ages respectively 32.20 ± 0.80 and 27.70 ± 0.75) with the parental age at birth of the first familial cases personally examined (average values for paternal and maternal ages respectively 28.98 ± 0.94 and 24.51 ± 0.91).

Mann-Whitney tests, applied to this data set, showed that both paternal and maternal ages at birth of isolated cases are significantly increased when compared to corresponding paternal and maternal ages at the birth of controls (respective test probability values $P_{\text{pat}} = 0.0108$ and $P_{\text{mat}} = 0.0043$), in spite of the differences between paternal and maternal ages of isolated and control cases being virtually the same (respectively 4.443 and 4.463 years; $P_{\text{pat}} = 0.6079$). As expected, extremely significant figures were obtained from the comparison between paternal and maternal ages in both groups ($P_{\text{iso}} = 0.0001$ and $P_{\text{mat}} = 0.0019$).

The effect of parental age can also be verified indirectly examining the position of the affected individual inside his or her sibship$^7$. If A is the rank of the isolated affected case in a sibship of size k with $h = 1 \leq k$ affected, the total expected number corresponding to the observed one $\Sigma A = 6L A$ is given by $3l(h(k+1))$, with a standard deviation $s.d. = \sqrt{\Sigma l(h(k+1))(k-h)}$. If $\Sigma A > 3l(h(k+1)) + 1.96$ s.d., one concludes that the affected ones tend to be born after the normal ones. Since for our set of data $6L A = 1056$ and $3l(h(k+1)) + 1.96$ s.d. = 982, we conclude that the effect of the order of birth in NF1 is statistically significant at the 5% level.

We also determined the fitness value of NF1 patients by comparing the average number of children of adult affected individuals with the corresponding average offspring number of a group of controls formed by their adult normal brothers and sisters. The approximate 95% confidence intervals for the average numbers of children per affected individual and per control were respectively $(2.467 < 3.005 < 3.590)$ and $(2.320 < 2.983 < 3.720)$, indicating that no differences in fitness values were detected. The application of basic principles of population genetics shows that the probability of a case of autosomal dominant condition being inherited is given by $P(h|af) = (1-s)(1+p(1-2s)) = (1-s)$, where s is the coefficient of selection of affected individuals. Therefore, about 50% of all cases of NF1 should originate from new mutations. We have no explanation for this discrepancy (also revealed by some other reports in the literature), but surely the subject merits further deepening.

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