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This project describes research in statistical methods that would be useful for statistical modelling and analysis of clinical data from NF1 and NF2 subjects. The statistical methods are classified into the areas:

(a) estimation of familial correlation for different types of data,
(b) assessment of multi-hit mutation models for incidence of tumours.

Some of the statistical methods to be developed are either new or partly new and require further research for computer software implementation. One goal of the project is to produce a software package for familial data analysis for different types of data, such as binary, count, censored survival data.

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Introduction

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Annual Report -body

Purpose of the project

(A) To develop statistical methods that can be used to characterize the phenotype of individuals with NF1 and NF2.

(B) To develop methods to elaborate on the standard two-hit model of tumour formation taking into account additional pathogenic factors and allelic differences for tumours in NF1 and NF2.

Objectives

The progress on the project's objectives are summarized below.

Objective 1. Develop statistical methods for interval-censored data, and obtain estimates of age of onset distributions for NF1 and NF2 features, using longitudinal information in the databases.

This objective is being postponed as we currently do not have enough longitudinal information in the databases.

Objective 2. Develop statistical methods for familial correlations for non-continuous and censored data, and obtain estimates of intraclass and interclass correlations for quantitative and binary traits in NF1 and NF2.

This objective has been progressing well. Software based on maximum likelihood and estimating equation methods and the multivariate probit and logit models have been implemented into code in the C/C++ programming languages for estimation of familial associations (latent correlations for probit, odds ratios for logit) for binary traits (e.g. presence/absence of a feature such as café-au-lait spots, plexiform neurofibromas, intertriginous freckling, Lisch nodules in NF1 subjects). The software was used in the analyses in Chapter 8 of Szudek's PhD thesis, which was defended in July 2001; a paper (Szudek et al.) which report on these findings has been submitted and is included in the appendix, for reference.

Latif's MSc thesis discusses an implementation of the GEE2 estimating equation method for the multivariate logit model. The main advantage of this approach is faster computations when the data include families of size 6 or more. Latif shows that estimates of regression coefficients and odds ratio are very similar (difference in second or third decimal place) for the maximum likelihood and GEE2 estimation methods. A manuscript summarizing these results is in draft form.

Y. Zhao, in continuing PhD research work, is studying theory for various estimating equation approaches that should lead to more reasonable computing time of familial associations for traits of the form of categorical or count data. Some results on NF2 familial associations based on a simple model for familial count data are given in a forthcoming paper by Zhao et al.

Aeschliman, in his MSc project work, has been analyzing the survival data for NF2 subjects, and developing a method for estimating the familial correlation of survival time. This will be included in the forthcoming paper by Baser et al. The complication is that survival time is a right censored variable (those who are still alive are right-censored). Aeschliman has also made a start on a computer program for familial correlations for quantitative traits that are right censored; this module would be added to our software package when completed.

Objective 3. Fit multi-hit mutation models for the incidence of NF2 and NF1 tumours by age, distinguish whether a two-hit or three-hit model provides a better fit to the data, and adapt the models to account for mutation type and other factors.

Two- and three-hit models for vestibular schwanomas were fit to data for NF2 subjects; this was the MSc thesis work of Woods. Since then, some modification have been done with different values that indicate the growth of Schwann cells. These results will appear in a forthcoming paper by Woods et al. Genotype-phenotype correlations have been reported in subjects with NF2 and a model that incorporates a subject's genotype has been fit. These results will appear in another manuscript.

Objective 4. Write C code to implement all of these statistical methods and provide a user-friendly interface for the code.

This is proceeding well. Software written in C/C++, developed in Unix/Linux, runs also in Windows with Cygnus/Gnuwin [see www.cygwin.com], the public domain version of Unix for Windows. The implementation of the interface currently is through a control file which specifies parameters and data files. A graphical user interface may be considered later, but this will not be straightforward as it is not easy to port Unix graphical interfaces to Windows.

The plan for the software is to allow for more different variable types. Familial associations are implemented by accounting for different relation types within families, such as sib-sib, parent-offspring, degree 2 relation, degree 3 relation etc. There is flexibility in that one can merge relation types such as relations of degree 2 or more, or make further splits such as mother-offspring and father-offspring in place of parent-offspring. The computer code based on GEE2 (see above) is being integrated into the software package.

The latest version of the software package can be obtained from the directory ftp://ftp.stat.ubc.ca/pub/hjoe/famil/

The summary according to the proposed time line of work is given below.

0-12 months :
- development of statistical theory for the simpler cases,
- coding into C programs and use on current NF1/NF2 databases

12-24 months :
- extension of theory to cover more general situations
- continuation of coding and data analysis
- presentation of preliminary results in technical papers and conferences

The theory for simplest cases has been mostly developed for objectives 2 and 3. The coding into C programs and use on current NF1/NF2 databases has been done for some of the methods. One manuscript has been submitted and a few others are near the submission stage. Some presentations have been or will be made at the 2000 and 2001 meetings of the American Society of Human Genetics.

24-36 months :
- writing more general publications,
- conversion of C code to a form with friendlier input instructions, so that a non-computer programmer can use the computer programs.

Key Research Accomplishments

- Comparison of two- and three-hit models for onset time of vestibular schwannomas for NF2 subjects..
- Start of software package for analysis of familial data of various types (binary, count, continuous, censored). The software written in the C/C++ programming languages, developed in Unix/Linux, runs also in Windows with Cygnus/Gnuwin.
- Application of the software package for estimating familial associations for NF1 clinical features, with adjustments for the age effect.

Reportable Outcomes

Theses

R. Woods. Models for the development of tumours in neurofibromatosis 2.
MSc thesis, Department of Statistics, University of British Columbia, August 2000.

J. Szudek. Analysis of variable expressivity in neurofibromatosis 1.
Ph.D. thesis, Department of Medical Genetics, University of British Columbia, July 2001.

A. H. Md. M. Latif. A comparison of methods for multivariate binary response with simulation studies.
MSc thesis, Department of Statistics, University of British Columbia, August 2001.

Scientific Poster Presentations at National or International Meetings

Palmer C, Joe H, Szudek J, Riccardi VM, Friedman JM: The development of cutaneous neurofibromas is influenced by familial and local factors in patients with neurofibromatosis 1 (NF1). Am J Hum Genet 67 (Suppl. 2): 132, 2000.

Szudek J, Joe H , Friedman JM: Familial aggregation of neurofibromatosis 1 (NF1) clinical features. Am J Hum Genet 67 (Suppl. 2): 211, 2000

Woods R, Joe H, Evans DGR, Baser ME, Friedman JM (2000). Extension of the two-hit hypothesis in neurofibromatosis 2 (NF2): effects of the mutant allele and prediction of the age of onset for both vestibular schwannomas. Am J Hum Genet 67 (Suppl. 2): 107, 2000.

Papers submitted for publication

Szudek J, Joe H, and Friedman JM (2001). Analysis of intra-familial phenotypic variation in neurofibromatosis 1 (NF1) Submitted to Am J Hum Genet, May 2001.

Papers near completion.

Woods R, Friedman JM, Evans DGR, Baser ME, and Joe H (2001). Exploring the `2-Hit Hypothesis' in NF2: Tests of 2-hit and 3-hit Models of Vestibular Schwannoma Development.

Zhao Y, Kumar RA, Baser ME, Evans DGR, Wallace A, Rouleau G, Kluwe L, Joe H, Friedman JM (2001). Intrafamilial correlation of clinical manifestations in neurofibromatosis type 2 (NF2).

Baser ME, Friedman JM, Wallace AJ, Ramsden RT, Aeschliman D, Joe H, Evans DGR (2001). Predictors of mortality in neurofibromatosis 2.

Conclusions

Conclusions:

The project is progressing well. For the models for familial data and estimation of familial associations (objective 2), future work will also consider various estimating equation approaches that are computationally faster; this methodology was not stated explicitly in the original proposal.

Appendix

Szudek J, Joe H, and Friedman JM (2001). Analysis of intra-familial phenotypic variation in neurofibromatosis 1 (NF1) Submitted for publication.

ANALYSIS OF INTRA-FAMILIAL PHENOTYPIC VARIATION IN NEUROFIBROMATOSIS 1 (NF1)

Running Title: Intra-familial variation in neurofibromatosis 1

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ABSTRACT

The relationship of genetic factors to variable expressivity in neurofibromatosis 1 (NF1) is poorly understood. We examined familial aggregation of NF1 features among different classes of affected relatives. Clinical information was obtained from the National NF Foundation International Database on 913 affected individuals in 373 families with 2 or more members with NF1. We used multivariate probit and multivariate normal regression to measure the associations between various classes of relatives for each of 12 clinical features of NF1 while simultaneously adjusting for covariates including related features, age and gender.

Statistically significant associations among relatives were found for café-au-lait spots, intertriginous freckling, neurofibromas, Lisch nodules, head circumference and stature but not for optic glioma, other neoplasms, seizures or scoliosis. Three distinct patterns were observed among the associations for familial features when compared between 1st and 2nd degree relatives and between sib-sib and parent-child pairs: 1) Head circumference and stature had similar associations for all three relationships; 2) Lisch nodules and café-au-lait spots had greater associations between 1st degree relatives than between 2nd degree relatives; and 3) Subcutaneous neurofibromas, plexiform neurofibromas, café-au-lait spots, and intertriginous freckling had greater associations between sibs than between parents and children. In addition, Lisch nodules, subcutaneous neurofibromas and cutaneous neurofibromas had greater associations between affected fathers and children than between affected mothers and children. These familial patterns suggest that the mutant NF1 allele, unlinked modifying genes, and the

normal NF1 allele may all be involved in the development of particular clinical features of NF1, but that the relative contributions vary for different features.

INTRODUCTION

Neurofibromatosis 1(NF1) is an autosomal dominant disease that affects about 1/3,500 people (Friedman 1999). NF1 can affect the skin, skeleton and nervous system and is characterized by highly variable expressivity (Riccardi 1992). Many disease features are progressive, but the rate of progression and the occurrence of serious manifestations vary greatly from one patient to another (Friedman and Riccardi 1999). This variability and the confounding effect of age have hindered efforts to characterize the relationship of genetic factors at the *NF1* locus or other loci to disease variability.

Mutation analysis of NF1 patients is difficult due to the large size of the *NF1* gene (335kb of genomic DNA) (Li et al. 1995), the existence of multiple unlinked pseudogenes and the large variety of mutational lesions (Viskochil 1999). The most effective single method for mutation analysis is the protein truncation test, but it detects mutations in only about 2/3 of patients (Heim et al. 1995; Park and Pivnick 1998). Messiaen et al. (2000) reported a mutation detection rate of greater than 90% in 67 NF1 probands, but this required a combination of protein truncation, heteroduplex, FISH, Southern blot and cytogenetic techniques.

More than 400 different constitutional *NF1* mutations have been reported (Korf 1999; Fahsold et al. 2000; Messiaen et al. 2000). In general, little evidence has been found of allele-phenotype correlations in NF1, although a more or less consistent phenotype occurs in association with deletions involving the entire *NF1* gene (Tonsgard et al. 1997; Dorschner et al. 2000). Similar clinical features have been observed among affected members of a few families with the NF1 variants Watson syndrome (Allanson et al.

1991), familial café-au-lait spots (Abeliovich et al. 1995) or familial spinal neurofibromas (Pulst et al. 1991; Poyhonen et al. 1997; Ars et al. 1998). This observation is consistent with an allele-phenotype correlation, but no consistent kind of *NF1* mutation has been found in families with these or other phenotypic variants. Affected members of a single family with typical NF1 often have quite different disease phenotypes, despite sharing an identical mutant *NF1* allele. Clearly, variation in the mutant *NF1* allele itself is insufficient to account for the variability of most disease features.

Mouse models for NF1 support the importance of other genetic factors in the development of disease features. Transgenic mice expressing the human T-lymphotropic virus type 1 *tax* gene develop dermal discrete neurofibromas and, occasionally, Lisch nodules (Hinrichs et al. 1987; Nerenberg et al. 1987; Green et al. 1992). The Tax *trans*-regulator represses *NF1* gene expression through a *cis*-acting element upstream of its transcriptional start site (Feigenbaum et al. 1996). Although repression by *tax* occurs in the absence of mutation at the *NF1* locus, transcriptional regulation of the normal or mutant alleles may affect disease pathogenesis in NF1 patients. $Nf1^{+/-}$ mice do not develop the any of the lesions characteristic of human NF1, but mice with inactivating mutations of both *Nf1* and *Trp53* on the same chromosome frequently develop astrocytomas, suggesting that there is biochemical interaction between the products of these two loci. Interestingly, the frequency of astrocytomas in $Nf1^{+/-}$ $Trp53^{+/-}$ cis double heterozygotes varies depending on genetic background (Reilly et al. 2000). These models provide evidence that genetic factors at other loci can affect the phenotype associated with *Nf1* mutations but are limited to only a few of NF1 disease features observed in humans.

Easton et al. (1993) studied the expressivity of NF1 in 175 affected members of 48 families and found statistically significant correlations for the number of café-au-lait spots, the number of dermal discrete neurofibromas and head circumference among affected relatives. Comparison of the strength of these correlations in relatives of different classes provides evidence for modifying genes influencing the number of café-au-lait spots. These results rely heavily on near-perfect concordance among 6 pairs of monozygotic twins and were not adjusted for the non-independence of multiple relative-pairs from the same family.

We have shown previously that several statistically significant associations exist between the occurrence of individual clinical features in 3067 unrelated probands with NF1 (Szudek et al. 1998; Szudek et al. 2000b). We also found significant associations in the occurrence of Lisch nodules, optic glioma, learning disability, macrocephaly and short stature in affected parent-child pairs (Szudek et al. 2000b), but made no attempt to adjust for the non-independence of multiple relative-pairs from the same family or for associations among clinical features in individuals in this preliminary study. We now extend our analysis to measure correlations of NF1 features among affected sibs, children and their mothers and fathers, and 2nd degree relatives using methods that take other clinical features and age into account and adjust for the non-independence of affected relatives. By comparing the correlations for relatives of various types, we provide evidence that genetic sources of variable expressivity are generally important in NF1 and vary for different clinical features.

SUBJECTS AND METHODS

Subjects

All patients in this study met the NIH diagnostic criteria for NF1 (NIH 1988; Gutmann et al. 1997). Data were obtained from the National NF Foundation International Database (NFDB) (Friedman et al. 1993) on 913 individuals from 373 families with 2 or more affected members, including 268 sib-sib, 373 parent-child and 74 2nd degree relative pairs.

For analysis of familiality, we selected 12 clinical features of NF1: café-au-lait spots, intertriginous freckling, Lisch nodules, cutaneous neurofibromas, subcutaneous neurofibromas, plexiform neurofibromas, head circumference, stature, seizures, scoliosis, optic glioma and neoplasms other than neurofibromas and optic gliomas ("other neoplasms"). Most of the features were identified by physical examination, and treated as binary variables. Café-au-lait spots were coded as "present" if the subject had 6 or more spots. Cutaneous or subcutaneous neurofibromas were coded as "present" if the subject had two or more lesions of the same type. Plexiform neurofibroma was coded as "present" if the subject had one or more lesions. Stature and head circumference were treated as continuous variables and standardised using population norms (Szudek et al. 2000a). Lisch nodules were diagnosed or excluded by a slit lamp examination. The presence or absence of optic glioma was determined by cranial MRI or CT examination. Only patients with definite presence or absence of a feature were considered in models involving that feature. The complete data set used in this study is available from the authors by request.

Statistical Methods

We included age as a covariate in all analyses. Age is one of the most important factors influencing the NF1 phenotype (Zöller et al. 1995). Many NF1 features, including Lisch nodules, subcutaneous neurofibromas, cutaneous neurofibromas, other neoplasms, intertriginous freckling, seizures and scoliosis have a higher prevalence in older patients (DeBella et al. 2000). We have shown previously that clinical features do not occur independently in NF1 patients, even after adjusting for the effect of age (Szudek et al. 1998; Szudek et al. 2000b). Therefore, we also controlled for the presence or absence of other associated features to minimize confounding in the present study.

Our general approach was to treat the features of NF1 as if each were a disorder occurring in a population affected with NF1. Familial aggregation of each binary feature among various classes of relatives was estimated using multivariate probit regression models, which assume that each of the binary dependent features reflects an underlying latent quantitative variable. Familial aggregation of continuous features (head circumference and stature) among various classes of relatives was estimated using multivariate normal regression models. The program MPROBIT was used for binary features and MVNFAM for continuous features. Two separate regressions were simultaneously applied to the feature being modelled (Joe 1997; Joe 2000). One regression accounted for covariates such as related features, interactions between related features (each represented by a distinct variable equal to the product of the two interacting features), age and gender. The second regression was used to measure latent correlation of the binary response variable between specific classes of relatives.

Alternatively, the second regression was used to measure correlation of the continuous response variable (head circumference or stature) between specific classes of relatives. MPROBIT and MVNFAM provide maximum likelihood estimates of the regression coefficients and standard errors for each covariate. The programmes also estimate the correlation coefficients and standard errors for the intra-familial relationships specified.

We used the results of a previous study of probands with NF1 from the NFDB (Szudek et al. 1998) to obtain appropriate functions for age (e.g. $e^{-age/4}$) and initial regression parameter estimates for covariates representing related features, interactions between related features and gender. Familial aggregation was assessed among sibs, parent-child pairs (including mother-child and father-child pairs separately) and 2nd degree relatives. Parameters and coefficients with 95% confidence intervals that excluded zero were deemed statistically significant. Standard errors and covariance matrices were used to test for differences between intra-familial correlation coefficients for different comparisons. For example, to test for a difference between sib-sib correlation and parent-child correlation we used the following formulas:

$$Z = \frac{r_{ss} - r_{pc}}{s} \quad \text{where} \quad s = \sqrt{(SE_{r_{ss}})^2 + (SE_{r_{pc}})^2 - 2\operatorname{cov}(r_{ss}, r_{pc})}$$

Z-scores were converted into p-values according to the standard normal distribution. We used one-tailed tests to compare correlations between 1st degree and 2nd degree relatives and between sib pairs and parent-child pairs because we had a prior expectation that correlations between sibs would be at least as strong as those between parents and children (Easton et al. 1993; Szudek et al. 2000b). We used two-tailed tests to compare mother-child correlations to father-child correlations.

RESULTS

We studied 913 individuals with NF1 from 373 families with two or more affected members. 91% of the individuals studied were White, 2% Asian, 1% Black, 1% Latin, and the remainder either of "other" or "unknown" origin. Table 1 shows the prevalences of each of the 12 NF1 clinical features in affected fathers, mothers and their affected children in the NFDB study sample and compares them to the prevalences in the sample used by Easton et al. (1993).

Familial aggregation among various classes of relatives was estimated using multivariate regression models. Table 2 shows the regression parameters and standard errors for the terms that were included in each model. The strength of association between the modelled feature and a covariate is measured by β. A unit increase in the value of the covariate means the modelled feature is $\exp(2\beta)$ times more likely to be present. For example, subjects with intertriginous freckling were $\exp(2\times.51)=2.8$ times more likely also to have café-au-lait spots than subjects of the same age and gender without intertriginous freckling. Also, subjects with intertriginous freckling *and* subcutaneous neurofibromas were $\exp(2\times(.51-.41+.61))=4.1$ times more likely to also have café-au-lait spots.

The parameter estimates for age were highly significant ($p<0.001$) for Lisch nodules, subcutaneous neurofibromas, cutaneous neurofibromas, and intertriginous freckling;

significant ($p<0.05$) for café-au-lait spots, head circumference, stature, optic gliomas and plexiform neurofibromas; and not significant ($p>0.05$) for other neoplasms, seizures and scoliosis. The parameter estimate for gender was not significant in any of the models. However, parameters that are not statistically significant on their own can still contribute to model interpretation and significance when other related features are also considered.

Table 3 shows the number of sib, parent-child (including mother-child and father child) and 2nd degree relative pairs used in each model. Subjects were included in a model only if the status ("presence" or "absence") of the modelled feature and all covariates was known.

Figure 1 shows the adjusted intrafamilial latent correlation coefficients and their 95% confidence intervals for each of the 12 features among all 913 relatives with NF1 from the 373 families studied. In these estimates, all relatives are treated the same regardless of relationship. Statistically significant positive intrafamilial correlations were observed for Lisch nodules, head circumference, subcutaneous neurofibromas, cutaneous neurofibromas, stature, café-au-lait spots and intertriginous freckling. Correlations for optic glioma, other neoplasms, seizures, scoliosis and plexiform neurofibromas, although positive, were not statistically different from zero. However, the number of individuals who had the latter features, especially optic glioma, other neoplasms, or seizures, was small, and the confidence intervals are very wide.

Figure 2 shows the adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 clinical features among 746 affected 1st degree relatives and among 148 affected 2nd degree relatives. MPROBIT failed to converge on correlation coefficients between 2nd degree relatives for optic glioma, other neoplasms, seizures or scoliosis because of the low frequency of these features and insufficient sample size. We did obtain correlation coefficients between 1st degree relatives for these features, but none was significantly different from zero. Statistically significant positive correlations between 1st degree relatives were found for 7 of the 8 other features listed in Figure 1. Significant positive correlations between 2nd degree relatives were also found for 4 of these 8 features. Significant negative correlations were not observed for any of the features. Correlations were significantly greater among 1st degree relatives than among 2nd degree relatives for Lisch nodules (p=0.0001) and café-au-lait spots (p=0.0004). Correlations among 1st degree relatives were not statistically different from correlations among 2nd degree relatives for head circumference (p=0.15), subcutaneous neurofibromas (p=0.06), cutaneous neurofibromas (p=0.49), stature (p=0.30), intertriginous freckling (p=0.07) or plexiform neurofibromas (p=0.11).

Figure 3 shows the adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 features among 268 affected sib pairs and among 373 affected parent-child pairs. Again, MPROBIT failed to converge on correlation coefficients between sibs or parent-child pairs for optic glioma, other neoplasms, seizures or scoliosis. Statistically significant positive correlations between sibs were found for all 8 features in Figure 3. Significant positive correlations between parents and children were found for 6 of the 8 features. Significant negative correlations were not observed for any of the features. Correlations were significantly greater between sibs than between parents and children for subcutaneous neurofibromas (p=0.04), café-au-lait spots (p=0.001), intertriginous freckling (p=0.03) and plexiform neurofibromas (p=0.02). Correlations between sibs were not statistically different from the correlations between parents and children for

Lisch nodules (p=0.40), head circumference (p=0.45), cutaneous neurofibromas (p=0.29), or stature (p=0.20).

Figure 4 shows the adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 features between 233 affected mother-child pairs and between 140 affected father-child pairs. Statistically significant positive correlations between mothers and children were found for 5 of the 8 features. Significant positive correlations between fathers and children were found for 6 of the 8 features. Significant negative correlations were not observed for any of the features in either relationship. Correlations between fathers and children are significantly greater than correlations between mothers and children for Lisch nodules (p=0.001), subcutaneous neurofibromas (p=0.0001) and cutaneous neurofibromas (p=0.02). Correlations do not differ significantly between father-child pairs and mother-child pairs for head circumference (p=0.85), stature (p=0.40), café-au-lait spots (p=0.62), intertriginous freckling (p=0.71) and plexiform neurofibromas (p=0.17).

DISCUSSION

Variable expressivity is a characteristic of many dominantly-inherited human genetic diseases and may have genetic or non-genetic causes. Possible genetic causes of variable expressivity include the effects of differences in the mutant allele, effects of the normal allele, and the effects of modifying genes. For example, analysis of phenotypic variation in von-Hippel-Lindau (VHL) disease has implicated unlinked modifying genes in the pathogenesis of ocular tumours (Webster et al. 1998), and the risk of ovarian cancer in *BRCA1* mutation carriers is modified by allelic variation at the unlinked *H-RAS* locus (Phelan et al. 1996). Our study was designed to evaluate the relative importance of various genetic mechanisms in the interfamilial and intrafamilial variability of NF1.

We analysed familial latent correlations for 10 NF1 clinical features and correlations for 2 NF1 clinical features, while adjusting for other related features, age and gender through statistical modelling. We found 7 of the features to have significant overall intra-familial correlations (Figure 1). We were also able to test for differences between correlations among various classes of relatives for 8 of the 12 features studied. Differences between various classes of relatives were found for 6 of the 7 features with significant overall intra-familial correlations (Figures 2-4).

Several features had significantly positive correlations among 2nd degree relatives, but none were significantly greater than the correlations for the same feature among 1st degree relatives (Figure 2). Similarly, several features had significantly positive correlations between parents and children, but none were greater than correlations for the same feature between sibs (Figure 3). The absence of significant negative correlations supports the statistical validity of our approach. One would expect to observe negative, as well as positive, correlations by chance when making multiple comparisons.

The NFDB draws its information from specialised clinics, so we were concerned about the representativeness of our sample. Furthermore, patients with unknown status of a feature were excluded from models involving that feature. Nevertheless, frequencies of features found among the familial cases used in this study (Table 1) are comparable to those found in another family study of variable NF1 expressivity (Easton et al. 1993). They are also comparable to the feature frequencies from two available population-based studies of NF1 patients (Samuelsson and Axelsson 1981; Huson et al. 1989).

Easton et al. (1993) studied 175 individuals with NF1 from 48 families, including 6 pairs of monozygotic twins, 76 pairs of sibs, 60 parent-offspring pairs, 54 2nd degree relative pairs and 43 3rd degree relative pairs. They examined 8 NF1 clinical features and found significant intrafamilial correlations for 3 quantitative variables: number of café-au-lait spots, number of cutaneous neurofibromas and head circumference. They also analysed 5 traits as binary variables, but these comparisons did not include adjustments for age. Furthermore, none of their analyses adjusted for the non-independence of multiple relative-pairs from the same family or of various clinical features.

Our sample size is 5 times larger, and we examined 12 clinical features, 6 of which are the same as Easton's. Also, we included associations between features as covariates in the familial analyses. Unlike Easton et al., we did not have counts of café-au-lait spots and dermal discrete neurofibromas, but Easton's quantitative investigations of these features complement our binary analyses nicely. Both studies found evidence of modifying genes on café-au-lait spots, but not on dermal discrete neurofibromas. In all, 10 of our 12 features were treated as binary variables – we had quantitative data only on stature and head circumference. Many of the clinical features of NF1 (and other diseases) are by nature binary, and ours is the first study to examine correlations for binary traits among different familial relationships while accounting for continuous covariates such as age. Similar methods have been used to study lens opacities (Framingham Eye Study, 1994) and liver cancer (Liang and Beaty 1991) in individuals who do not have NF1, but we may be the first to study an autosomal dominant disease in this manner.

Although this is by far the largest group of NF1 families ever studied, we only had 74 pairs of 2nd degree relatives. Models for most features used even fewer 2nd degree relatives because the data were incomplete. Subjects were included in a model only if the status of the modelled feature and all covariates was known (Table 3). These relatively small sample sizes are reflected in the wide 95% confidence intervals for the correlation coefficients among 2nd degree relatives (Figure 2). Furthermore, statistical techniques are less reliable for smaller sample sizes, so we must attach an additional note of caution to the point estimates for the correlation coefficients between 2nd degree relatives, particularly for Lisch nodules, head circumference, stature and intertriginous freckling, in which the analysis included 35 or fewer pairs of 2nd degree relatives (Table 3).

The most important confounding factor in familial analyses of NF1 is age. Many disease features are more prevalent in older NF1 patients (Cnossen et al. 1998), and, if not appropriately controlled, age might produce a correlation between affected relatives of similar age (e.g., sibs) or obscure a correlation between relatives of very different ages (e.g., parents and children). Our multivariate models minimise the confounding effect of age, but they may not eliminate it completely. The covariate representing age was significant in models for most features, but it is possible that a residual age effect is contributing to the observed differences between sib and parent-child pairs for features such as subcutaneous neurofibromas and intertriginous freckling that become more prevalent with age (Figure 3). Age is less likely to influence the intrafamilial correlations for café-au-lait spots or plexiform neurofibromas, which, when considered as discrete variables, occur with a relatively stable frequency with age (Riccardi 1992; DeBella et al. 2000).

We used one-tailed tests for 1st degree vs. 2nd degree and sib-sib vs. parent-child comparisons. Several of the results just reach a level of nominal statistical significance using one-tailed z-tests, and several others fall only a little short of doing so. Clearly these results require independent confirmation in future studies.

Lisch nodules and café-au-lait spots had significantly higher correlations among 1st degree relatives than among 2nd degree relatives. Higher correlations for 1st than 2nd degree relatives would be expected for effects produced by modifying genes at unlinked loci but might also result from environmental factors that are more likely to be shared among closer relatives. However, it is hard to imagine what environmental factors could contribute to the development of Lisch nodules. No family studies have previously been done on Lisch nodules, and factors contributing to their development are unknown. Our observations are consistent with the effect of a modifying gene on the pathogenesis of Lisch nodules.

Easton et al. (1993) found a higher correlation for café-au-lait spots between MZ twins than between sibs, suggesting the effect of a genetic locus or loci in addition to

NF1. Our findings of a strong correlation for café-au-lait spots in 1st degree relatives but no correlation among 2nd degree relatives are consistent with this interpretation.

Lisch nodules are melanocytic hamartomas that arise in iris tissue (Perry and Font 1982). Café-au-lait spots are pigmented macules composed of melanocytes with abnormally large pigment particles (Fitzpatrick 1981). Lisch nodules and café-au-lait spots share an origin from neural crest-derived tissue, but this is also true of some other lesions characteristic of NF1, including neurofibromas of all types and intertriginous freckling (Bolande 1981). We previously reported an association between the occurrence of Lisch nodules and café-au-lait spots in individual NF1 patients (Szudek et al. 2000b), but intertriginous freckling was also associated – a feature that shows no indication of a stronger familial correlation among 1st degree than 2nd degree relatives (Figure 2). If the development of Lisch nodules and café-au-lait spots is influenced by modifying genes, it is unclear what the nature of these modifying factors is or whether they are the same or different for these two features.

Intertriginous freckling, subcutaneous neurofibromas, plexiform neurofibromas and café-au-lait spots had higher correlations between sibs than between parents and children. Both sib pairs and parent-child pairs are 1st degree relatives who would be expected to share a similar proportion of non-allelic modifying genes, so the differences we observed in these correlations are unlikely to result from effects of modifying genes. Nevertheless, Easton et al. (1993) found that concordance for dermal discrete neurofibromas (which include subcutaneous neurofibromas) between monozygotic twins was much higher than between sibs, an observation that suggests the involvement of a genetic factor. Affected sibs would be expected to share the same normal *NF1* allele by descent half of the time, but parent-child pairs rarely would. Effects of functional polymorphisms of the normal *NF1* allele might explain a higher correlation of these features among sib pairs than among parent-child pairs, but no direct evidence is available on this possibility, and the frequency of functional polymorphisms of the *NF1* locus is unknown. Another possible explanation is differences in environmental factors that are more likely to be shared among sibs than between a parent and child.

Intertriginous freckling, subcutaneous neurofibromas, plexiform neurofibromas and café-au-lait spots all share an origin from neural crest-derived cells. We found that café-au-lait spots and intertriginous freckling tended to occur together in individual NF1 patients, and so did cutaneous, subcutaneous, and plexiform neurofibromas, but associations were not seen between the features in these two groups (Szudek et al. 1998). In the present study, we did not find a stronger correlation for cutaneous neurofibromas in sibs than in parent-child pairs, as we did for subcutaneous and plexiform neurofibromas (Figure 3). Intertriginous freckling occurs in skin folds, and local environmental factors may play a role in the development of such freckling (Riccardi 1992). There is anecdotal evidence that subcutaneous neurofibromas may also develop as a result of trauma (Riccardi 1990). This hypothesis has not been tested formally, and it seems unlikely to account for the development of congenital diffuse plexiform neurofibromas. In any case, it is unclear why factors like cutaneous trauma would be more similar in sibs than in parent-child pairs.

Lisch nodules, subcutaneous neurofibromas, and cutaneous neurofibromas had higher correlations between affected fathers and children than between affected mothers and children (Figure 4). Our sample included twice as many mother-child pairs as father-child pairs, so we were concerned about ascertainment bias – the possibility that only severely affected father-child pairs tend to be seen in the NF clinics that contributed data to the NNFF International Database. However, the frequencies of all features studied were similar in affected fathers as in affected mothers (Table 1).

Shared environment is unlikely to be the sole cause of associations between parents and children, due to large differences in age. It is also unlikely that shared environment is responsible for the difference in correlations between mother-child and father-child pairs. Likewise, a multifactorial influence with a more extreme threshold for males than for females cannot explain the observations for these features. Gender is not a significant predictive factor in any of our models (Table 2), and feature frequencies among affected children of affected fathers are similar to those among affected children of affected mothers (Table 1). Parent-of-origin effects on severity of NF1 have been suggested (Miller and Hall 1978; Hall 1981), but most studies do not support this possibility (Riccardi and Wald 1987; Huson et al. 1989). One study found a male predominance among NF1 patients with pseudarthrosis but no significant parent-of-origin effect (Stevenson et al. 1999). Our findings are consistent with a parent-of-origin effect on the strength of the parent-child correlation rather than with a more severe phenotype in affected offspring of parents of one gender when compared to affected offspring of parents of the other gender. Similar parent-child aggregation patterns have been reported for body mass index (Friedlander et al. 1988) and blood pressure (Hurwich et al. 1982), but they are unprecedented in NF1. Male-to-male inheritance is unlikely since gender is not a significant factor in any of our models (Table 2) and father-son concordance for Lisch nodules, subcutaneous neurofibromas, cutaneous neurofibromas is the same as father-daughter concordance, which argues against a Y-linked factor. We do not know of a genetic mechanism that can explain this phenomenon in NF1 or for body mass index or blood pressure.

Head circumference and stature had similar correlations for all relationships. This suggests that the mutant *NF1* allele itself is most important in determining these correlations. Easton et al. (1993) also found evidence of the importance of the mutant allele in head circumference. The distributions of head circumference and stature in NF1 patients are unimodal (Szudek et al. 2000a), but both NF1 distributions are shifted relative to unaffected norms, suggesting that head circumference and stature are affected to a degree in all NF1 patients. Taken together with the results of the present study, it appears that the magnitude of this effect depends, at least partly, on the mutant *NF1* allele. The mutant *NF1* genotype also has a very strong effect on the phenotypic manifestations in patients with Watson syndrome (Allanson et al. 1991) or deletions of the whole *NF1* locus (Tonsgard et al. 1997; Dorschner et al. 2000).

The patterns of familial correlations shown here suggest that genetic factors involved in determining the occurrence of various clinical features of NF1 vary depending on the feature. In some instances, the mutant *NF1* allele may be most important. In other instances, the effects of the normal *NF1* allele or of unlinked modifying genes may predominate. More than one genetic factor may be involved, and the relative importance of various genetic and non-genetic effects may vary for different features.

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REFERENCES

Abeliovich D, Gelman-Kohan Z, Silverstein S, Lerer I, Chemke J, Merlin S, Zlotogora J (1995) Familial café au lait spots: a variant of neurofibromatosis type 1. J Med Genet 32:985-986

Allanson J, Upadhaya M, Watson G, Partington M, MacKenzie A, Lahey D, MacLeod H, Sarfarazi M, Broadhead W, Harper P, Huson S (1991) Watson syndrome: Is it a subtype of type 1 neurofibromatosis? J Med Genet 28:752-756

Ars E, Kruyer H, Gaona A, Casquero P, Rosell J, Volpini V, Serra E, Lázaro C, Estavill X (1998) A clinical variant of neurofibromatosis type 1: Familial spinal neurofibromatosis with a frameshift mutation in the NF1 gene. Am J Hum Genet 62:834-841

Bolande R (1981) Neurofibromatosis - the quintessential neurocristopathy: Pathogenic concepts and relationships. Adv Neurol 29:67-75

Cnossen M, de Goede-Bolder A, van den Broek K, Waasdorp C, Oranje A, Stroink H, Simonsz H, van den Ouweland A, Halley D, Niermeijer M (1998) A prospective 10 year follow up study of patients with neurofibromatosis type 1. Arch Dis Child 78:408-412

DeBella K, Szudek J, Friedman JM (2000) Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics 105:608-14

Dorschner MO, Sybert VP, Weaver M, Pletcher BA, Stephens K (2000) NF1 microdeletion breakpoints are clustered at flanking repetitive sequences. Hum Mol Genet 9:35-46

Easton D, Ponder M, Huson S, Ponder B (1993) An analysis of variation in expression of neurofibromatosis (NF) type I (NFI): Evidence for modifying genes. Am J Hum Genet 53:305-313

Fahsold R, Hoffmeyer S, Mischung C, Gille C, Ehlers C, Kucukceylan N, Abdel-Nour M, Gewies A, Peters H, Kaufmann D, Buske A, Tinschert S, Nurnberg P (2000) Minor lesion mutational spectrum of the entire NF1 gene does not explain its high mutability but points to a functional domain upstream of the GAP-related domain. Am J Hum Genet 66:790-818

Feigenbaum L, Fujita K, Collins FS, Jay G (1996) Repression of the NF1 gene by Tax may expain the development of neurofibromas in human T-lymphotropic virus type 1 transgenic mice. J Virol 70:3280-5

Fitzpatrick TB (1981) Melanin synthesis pathways in the pathogenesis of neurofibromatosis. Adv Neurol 29:209-11

Framingham Eye Study (1994) Familial aggregation of lens opacities: the Framingham Eye Study and the Framingham Offspring Eye Study. Am J Epidemiol 140:555-64

Friedlander Y, Kark JD, Kaufmann NA, Berry EM, Stein Y (1988) Familial aggregation of body mass index in ethnically diverse families in Jerusalem. The Jerusalem Lipid Research Clinic. Int J Obes 12:237-47

Friedman J, Greene C, Birch P, and the NNFF International Database P (1993) National Neurofibromatosis Foundation International Database. Am J Med Genet 45:88-91

Friedman J, Riccardi V (1999) Clinical and epidemiological features. In: Friedman J, Gutmann D, MacCollin M, Riccardi V (eds) Neurofibromatosis : phenotype, natural history, and pathogenesis. Johns Hopkins University Press, Baltimore, pp 29-86

Friedman JM (1999) Epidemiology of neurofibromatosis type 1. Am J Med Genet 89:1-6

Green JE, Baird AM, Hinrichs SH, Klintworth GK, Jay G (1992) Adrenal medullary tumors and iris proliferation in a transgenic mouse model of neurofibromatosis. Am J Pathol 140:1401-10

Gutmann D, Aylsworth A, Carey J, Korf B, Marks J, Pyeritz R, Rubenstein A, Viskochil D (1997) The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 278:51-57

Hall J (1981) Possible maternal and hormonal factors in neurofibromatosis. Adv Neurol 29:125-131

Heim RA, Kam-Morgan LN, Binnie CG, Corns DD, Cayouette MC, Farber RA, Aylsworth AS, Silverman LM, Luce MC (1995) Distribution of 13 truncating mutations in the neurofibromatosis 1 gene. Hum Mol Genet 4:975-81

Hinrichs SH, Nerenberg M, Reynolds RK, Khoury G, Jay G (1987) A transgenic mouse model for human neurofibromatosis. Science 237:1340-3

Hurwich BJ, Rosner B, Nubani N, Kass EH, Lewitter FI (1982) Familial aggregation of blood pressure in a highly inbred community, Abu Ghosh, Israel. Am J Epidemiol 115:646-56

Huson S, Compston D, Clark P, Harper P (1989) A genetic study of von Recklinghausen neurofibromatosis in south east Wales: I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 26:704-711

Joe H (1997) Multivariate models and dependence concepts Monographs on statistics and applied probability ; 73. Chapman & Hall, London ; New York, pp xviii, 399

Joe H (2000) Programs for multivariate binary (logit/probit) models

Korf B (1999) NNFF International NF1 Genetic Analysis Consortium Mutation Summary Data. National Neurofibromatosis Foundation

Li Y, O'Connell P, Breidenbach HH, Cawthon R, Stevens J, Xu G, Neil S, Robertson M, White R, Viskochil D (1995) Genomic organization of the neurofibromatosis 1 gene (NF1). Genomics 25:9-18

Liang KY, Beaty TH (1991) Measuring familial aggregation by using odds-ratio regression models. Genet Epidemiol 8:361-70

Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F, Paepe AD (2000) Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. Hum Mutat 15:541-55

Miller M, Hall J (1978) Possible maternal effect on severity of neurofibromatosis. Lancet 2:1071-1073

Nerenberg M, Hinrichs SH, Reynolds RK, Khoury G, Jay G (1987) The tat gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. Science 237:1324-9

NIH (1988) Neurofibromatosis: Conference statement. National Institutes of Health Consensus Development Conference. Arch Neurol 45:575-8

Park VM, Pivnick EK (1998) Neurofibromatosis type 1 (NF1): a protein truncation assay yielding identification of mutations in 73% of patients. J Med Genet 35:813-20

Perry H, Font R (1982) Iris nodules in von Recklinghausen's neurofibromatosis: Electron microscopic confirmation of their melanocytic origin. Arch Ophthalmol 100:1635-1640

Phelan CM, Rebbeck TR, Weber BL, Devilee P, Ruttledge MH, Lynch HT, Lenoir GM, Stratton MR, Easton DF, Ponder BA, Cannon-Albright L, Larsson C, Goldgar DE, Narod SA (1996) Ovarian cancer risk in BRCA1 carriers is modified by the HRAS1 variable number of tandem repeat (VNTR) locus. Nat Genet 12:309-11

Poyhonen M, Leisti E-L, Kytölä S, Leisti J (1997) Hereditary spinal neurofibromatosis: A rare form of NF1? J Med Genet 34:184-187

Pulst S, Riccardi V, Fain P, Korenberg J (1991) Familial spinal neurofibromatosis: Clinical and DNA linkage analysis. Neurology 41:1923-1927

Reilly KM, Loisel DA, Bronson RT, McLaughlin ME, Jacks T (2000) Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat Genet 26:109-13

Riccardi V (1990) The potential role of trauma and mast cells in the pathogenesis of neurofibromas. In: Ishibashi Y, Hori Y (eds) Tuberous sclerosis and neurofibromatosis : epidemiology, pathophysiology, biology, and management. Elsevier, Amsterdam, pp 167-190

Riccardi V (1992) Neurofibromatosis: Phenotype, natural history, and pathogenesis. The Johns Hopkins University Press, Baltimore

Riccardi V, Wald J (1987) Discounting an adverse maternal effect on severity of neurofibromatosis. Pediatrics 79:386-393

Samuelsson B, Axelsson R (1981) Neurofibromatosis: A clinical and genetic study of 96 cases in Gothenburg, Sweden. Acta Dermatovenereolog Suppl 95:67-71

Stevenson DA, Birch PH, Friedman JM, Viskochil DH, Balestrazzi P, Boni S, Buske A, Korf BR, Niimura M, Pivnick EK, Schorry EK, Short MP, Tenconi R, Tonsgard JH, Carey JC (1999) Descriptive analysis of tibial pseudarthrosis in patients with neurofibromatosis 1. Am J Med Genet 84:413-9

Szudek J, Birch P, Friedman JM (2000a) Growth in north american white children with neurofibromatosis 1 (NF1). J Med Genet 37:933-8

Szudek J, Birch P, Riccardi VM, Evans DG, Friedman JM (2000b) Associations of clinical features in neurofibromatosis 1 (NF1). Genet Epidemiol 19:429-39

Szudek J, Evans D, Friedman J (1998) Logistic regresssive models of neurofibromatosis 1 (NF1) clinical features American Society of Human Genetics Annual Meeting. Am J Hum Genet, Baltimore

Tonsgard J, Yalavarthi K, Cushner S, Short M, Lindgren V (1997) Do NF1 gene deletions result in a characteristic phenotype? Am J Med Genet 73:80-86

Viskochil D (1999) The structure and function of the NF1 Gene: molecular pathophysiology. In: Friedman J, Gutmann D, MacCollin M, Riccardi V (eds) Neurofibromatosis : phenotype, natural history, and pathogenesis. Johns Hopkins University Press, Baltimore, pp 119-141

Webster AR, Richards FM, MacRonald FE, Moore AT, Maher ER (1998) An analysis of phenotypic variation in the familial cancer syndrome von Hippel-Lindau disease: evidence for modifier effects. Am J Hum Genet 63:1025-35

Zöller M, Rembeck B, Åkesson H, Angervall L (1995) Life expectancy, mortality and prognostic factors in neurofibromatosis type 1: A twelve-year follow-up of an epidemiological study in Göteborg, Sweden. Acta Derm Venerol (Stockh) 75:136-140

Table 1. Number and percentage of subjects from the NFDB and from the study by Easton et al. with various NF1 features. Features not considered by Easton et al. have empty cells in the last two columns.

	NFDB										Easton et al.	
Feature	**Affected Fathers**		**Affected Mothers**		**Affected Children of Affected Fathers**		**Affected Children of Affected Mothers**		**All Affected Relatives**		**All Affected Relatives**	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Lisch nodules	63	(81%)	101	(80%)	64	(63%)	101	(51%)	409	(60%)		
Café-au-lait macules	67	(68%)	125	(73%)	115	(79%)	181	(74%)	657	(75%)	129	(88%)
Cutaneous neurofibromas	78	(80%)	119	(69%)	37	(25%)	67	(27%)	355	(40%)	121	(76%)
Optic glioma	4	(13%)	2	(4%)	8	(14%)	14	(15%)	45	(15%)	9	(5%)
Subcutaneous neurofibromas	60	(61%)	99	(59%)	31	(21%)	58	(23%)	291	(33%)		
Intertriginous freckling	79	(83%)	147	(88%)	112	(78%)	191	(78%)	699	(80%)		
Seizures	7	(7%)	12	(7%)	8	(5%)	16	(6%)	58	(6%)	12	(7%)
Plexiform neurofibromas	24	(24%)	35	(20%)	25	(18%)	44	(18%)	176	(20%)	37	(21%)
Scoliosis	7	(8%)	8	(6%)	29	(22%)	32	(14%)	96	(12%)	27	(16%)
Other neoplasms	4	(4%)	11	(6%)	4	(3%)	7	(3%)	33	(4%)		

Table 2. Summary of regressions in multivariate models for 12 clinical NF1 features. The 1st column lists the 12 modelled features. The 2nd–4th columns show the covariates and their regression parameter estimates (β) with standard errors (SE) used in each model. β_0 is the intercept in the model equation. Each regression accounts for covariates such as related features, interactions between related features, age and gender. Interactions are depicted by features separated by an "*" and their values equal the product of the two interacting features.

Modelled Feature	**Intercept and Covariates**	β	SE
Lisch nodules	β_0	.65	(.08)
	Age	-3.55	(.32)
	Male gender	-.01	(.08)
	Café-au-lait spots	.23	(.15)
	Cutaneous neurofibromas	.44	(.20)
	Café-au-lait spots * Cutaneous neurofibromas	-.09	(.22)
Café-au-lait spots	β_0	.28	(.14)
	Age	-.66	(.25)
	Male gender	.03	(.09)
	Intertriginous freckling	.51	(.12)
	Subcutaneous neurofibromas	-.41	(.26)
	Intertriginous freckling* Subcutaneous neurofibromas	.61	(.28)
Head circumference	β_0	-.99	(.10)
	Age	.62	(.21)
	Male gender	-.09	(.31)
	Lisch nodules	-.06	(.36)
	Optic glioma	.56	(.44)
	Stature	.34	(.04)
	Neoplasms	.10	(.75)
Cutaneous neurofibromas	β_0	-1.62	(.11)
	Age	-5.56	(.36)
	Male gender	.01	(.10)
	Subcutaneous neurofibromas	.62	(.11)
	Plexiform neurofibromas	.36	(.12)
Stature	β_0	-.62	(.09)
	Age	-.82	(.31)
	Male gender	-.03	(.09)
	Head circumference	.04	(.01)
Optic glioma	β_0	-1.02	(.13)
	Age	.72	(.57)
	Male gender	.06	(.17)
	Plexiform neurofibromas	.01	(.37)
	Head circumference	.19	(.07)
	Neoplasms	.55	(.49)

Table 2 (continued)

Modelled Feature	**Intercept and Covariates**	β	SE
Subcutaneous neurofibromas	β_0	-1.72	(.12)
	Age	-3.78	(.35)
	Male gender	-.04	(.08)
	Café-au-lait spots	.43	(.11)
	Cutaneous neurofibromas	.73	(.13)
	Plexiform neurofibromas	.52	(.17)
	Intertriginous freckling * Plexiform neurofibromas	-.24	(.23)
Intertriginous freckling	β_0	.49	(.15)
	Age	-1.58	(.30)
	Male gender	-.23	(.12)
	Café-au-lait spots	.52	(.14)
	Subcutaneous neurofibromas	-.18	(.27)
	Lisch nodules	.55	(.14)
	Café-au-lait spots * Subcutaneous neurofibromas	.62	(.33)
Seizures	β_0	-1.43	(.11)
	Age	-.88	(.65)
	Male gender	-.04	(.15)
Plexiform neurofibromas	β_0	-1.11	(.11)
	Age	-.88	(.38)
	Male gender	.07	(.09)
	Subcutaneous neurofibromas	.46	(.16)
	Cutaneous neurofibromas	.37	(.14)
	Subcutaneous * Cutaneous neurofibromas	-.21	(.22)
Scoliosis	β_0	-1.11	(.09)
	Age	-.57	(.34)
	Male gender	-.02	(.11)
Other neoplasms	β_0	-.95	(.23)
	Age	-4.07	(2.11)
	Male gender	-.06	(.21)
	Lisch nodules	-.55	(.25)
	Optic glioma	.32	(.31)

Table 3. Number of relatives used in multivariate probit models for 12 clinical NF1 features. The 1st column lists the 12 modelled features. The 2nd-5th columns show the number of affected sib, mother-child, father-child and 2nd degree relative pairs for which the status of the modelled feature and covariates in Table 2 is known.

Modelled Feature	**Sib Pairs**	**Mother-Child Pairs**	**Father-Child Pairs**	**2° Relative Pairs**
Lisch nodules	192	159	79	35
Café-au-lait macules	248	210	129	69
Head circumference	103	64	37	24
Cutaneous neurofibromas	264	224	131	69
Stature	103	64	37	24
Optic glioma	55	37	26	4
Subcutaneous neurofibromas	253	220	131	69
Intertriginous freckling	179	148	75	35
Seizures	268	233	140	74
Plexiform neurofibromas	264	224	131	69
Scoliosis	228	191	131	53
Other neoplasms	47	33	20	3

Figure 1: Adjusted intrafamilial latent correlation coefficients and their 95% confidence intervals for each of the 12 features among all 913 relatives with NF1 from the 373 families studied. In these estimates, all relatives are treated the same regardless of relationship.

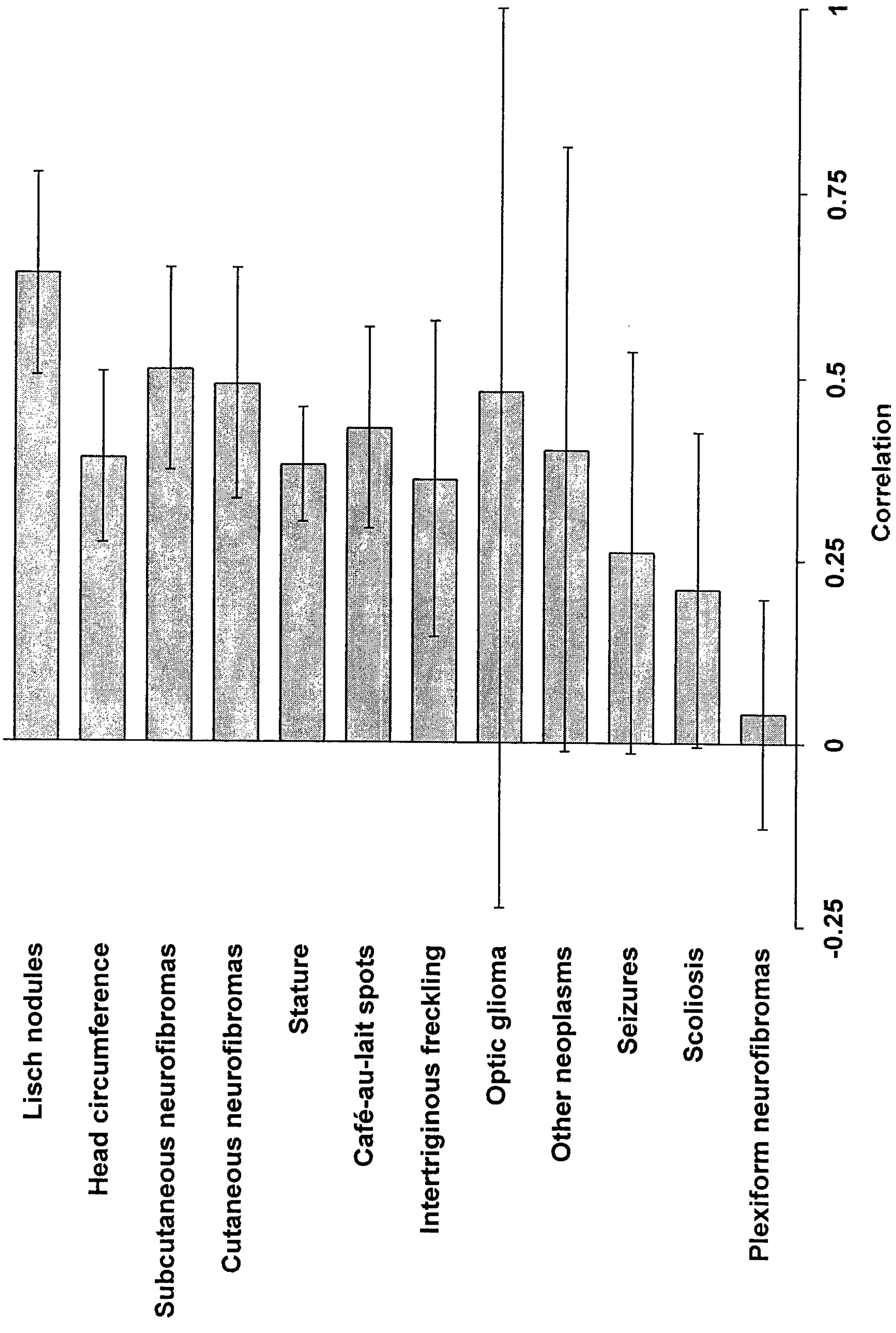

Lisch nodules
Head circumference
Subcutaneous neurofibromas
Cutaneous neurofibromas
Stature
Café-au-lait spots
Intertriginous freckling
Optic glioma
Other neoplasms
Seizures
Scoliosis
Plexiform neurofibromas
-0.25
0
0.25
0.5
0.75
1
Correlation

Figure 2: Adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 clinical features among 746 affected 1st degree relatives and among 148 affected 2nd degree relatives. A star indicates a significant difference between the correlation coefficients of the two classes being compared. MPROBIT failed to converge on correlation coefficients between 2nd degree relatives for optic glioma, other neoplasms, seizures or scoliosis because of the low frequency of these features and insufficient sample size.

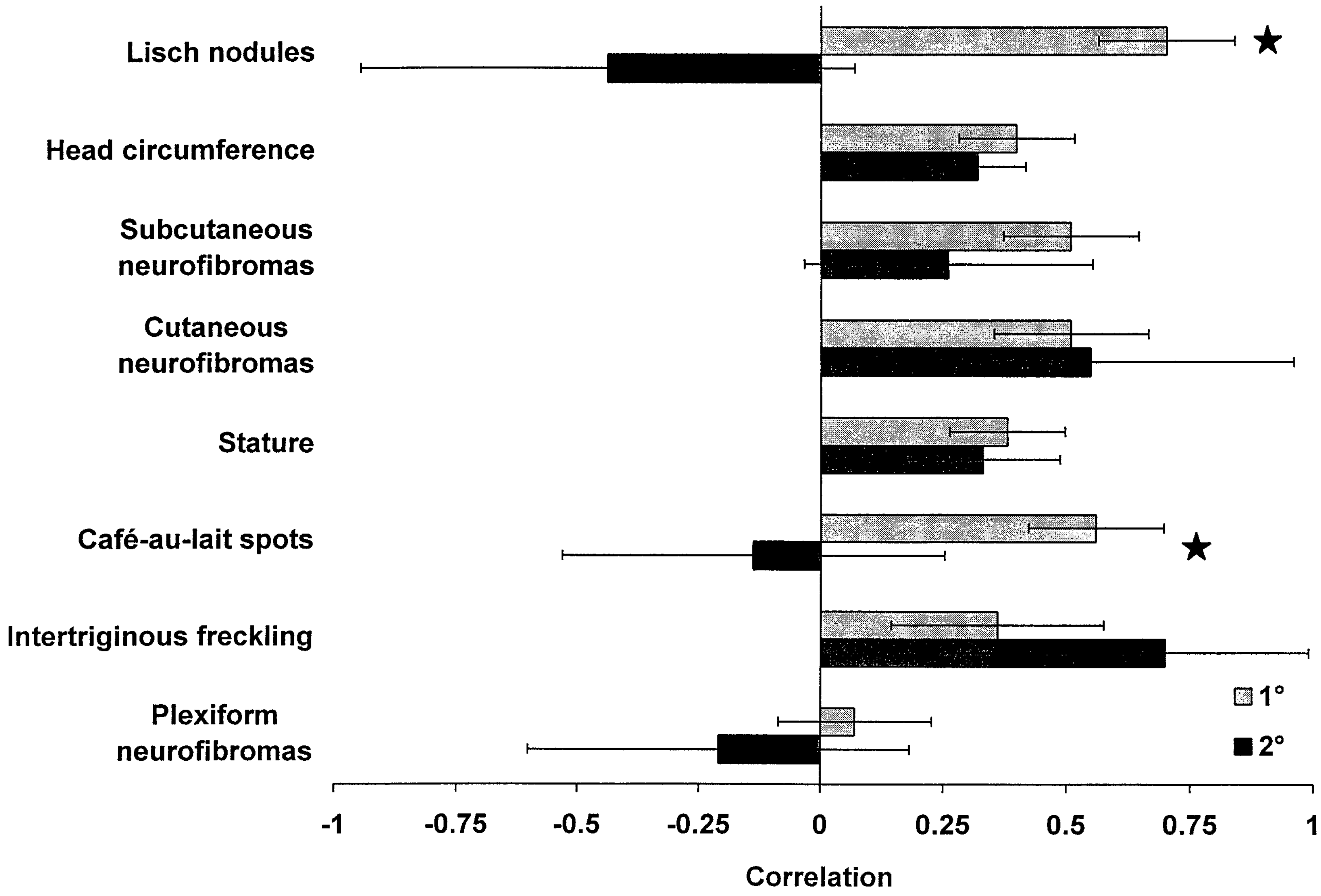
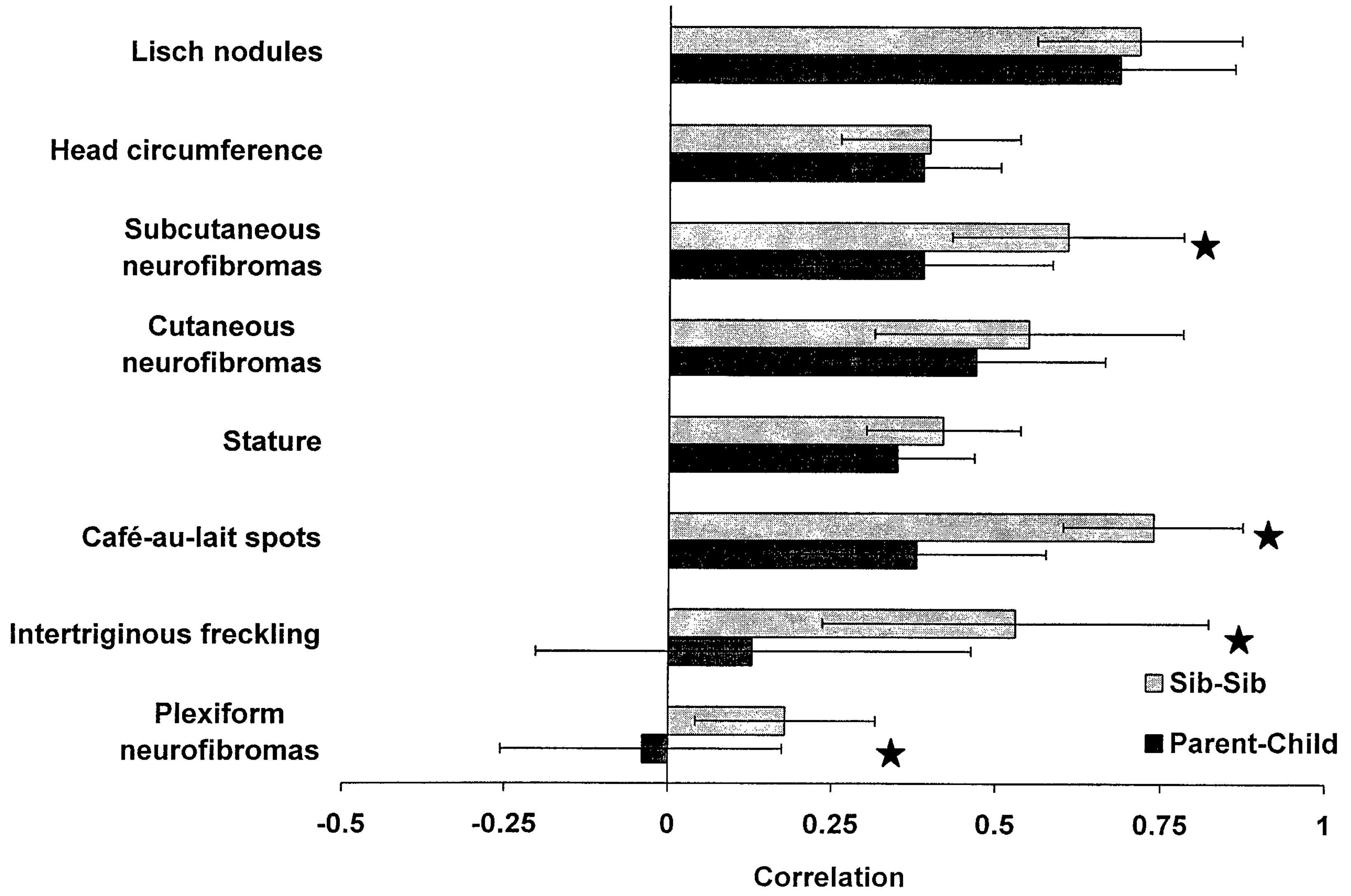

Lisch nodules
Head circumference
Subcutaneous neurofibromas
Cutaneous neurofibromas
Stature
Café-au-lait spots
Intertriginous freckling
Plexiform neurofibromas
★
★
▨ 1°
■ 2°
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1
Correlation

Figure 3: Adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 features among 268 affected sib pairs and among 373 affected parent-child pairs. A star indicates a significant difference between the correlation coefficients of the two classes being compared. MPROBIT failed to converge on correlation coefficients between sibs or parent-child pairs for optic glioma, other neoplasms, seizures or scoliosis.


Lisch nodules
Head circumference
Subcutaneous neurofibromas
Cutaneous neurofibromas
Stature
Café-au-lait spots
Intertriginous freckling
Plexiform neurofibromas
★
▨ Sib-Sib
■ Parent-Child
-0.5
-0.25
0
0.25
0.5
0.75
1
Correlation

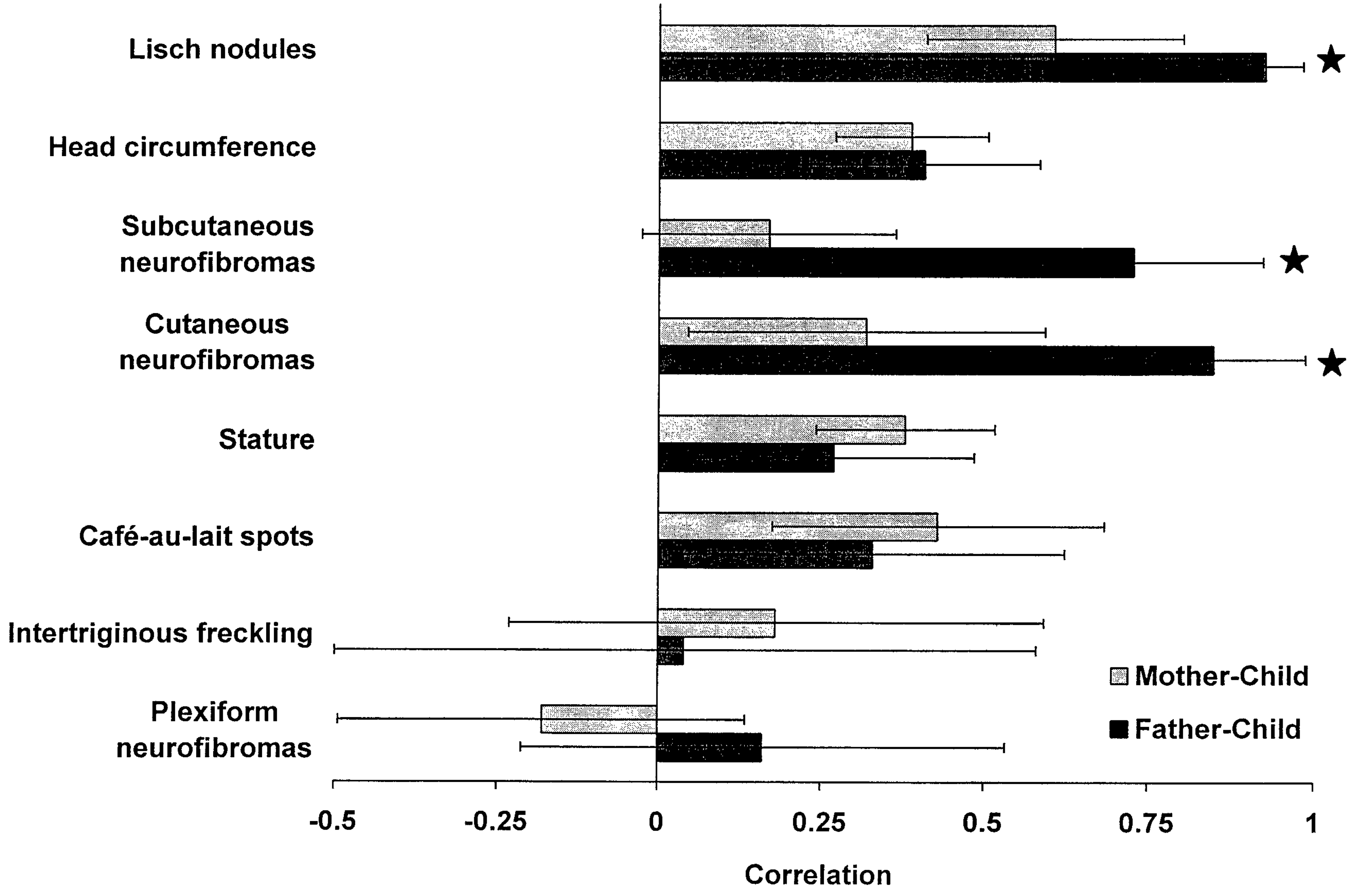

Lisch nodules
Head circumference
Subcutaneous neurofibromas
Cutaneous neurofibromas
Stature
Café-au-lait spots
Intertriginous freckling
Plexiform neurofibromas
★
★
★
▨ Mother-Child
■ Father-Child
-0.5
-0.25
0
0.25
0.5
0.75
1
Correlation

Figure 4: Adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 features between 233 affected mother-child pairs and between 140 affected father-child pairs. A star indicates a significant difference between the correlation coefficients of the two classes being compared.