suggests that integrin polymorphisms might be an important new focus for studies on genetic risk factors for pre-eclampsia.

- This study investigated the role of two thrombophilic gene polymorphisms, prothrombin 20210G>A and the 98C>T variation in the β3 integrin glycoprotein IIIa (GPIIIa), as risk factors for pre-eclampsia in an East Anglian cohort of 356 affected women.
- In contrast to an earlier report, we found no excess of carriers for the prothrombin 20210A variant in our cohort. However, for the *GPIIIa* 98C>T polymorphism there was an excess of 98T homozygotes in our pre-eclampsia group (18/356) versus controls (1/200, $p<0.01$). The odds ratio for 98T homozygotes was 11.3 (95% CI 1.5-85.5) and for carriage of at least one T98 allele, 1.4 (95% CI 1.0-2.1).
- These data suggest that carriage of the 98T polymorphism of *GPIIIa* may be a previously unidentified risk factor for pre-eclampsia.

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Cystic fibrosis patients with the 3272-26A>G splicing mutation have milder disease than F508del homozygotes: a large European study

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EDITOR—Cystic fibrosis (CF, MIM 219700) is a common, severe, autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene cloned in 1989.[1–3] The disease, characterised by chronic lung disease which is the main cause of morbidity and mortality, pancreatic dysfunction, raised electrolyte levels in sweat, and male infertility, is caused by altered chloride (Cl^-) secretion across the apical membrane of epithelial cells.[4] There

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is, however, substantial variability in the clinical manifestations affecting the various organs.[4 5]

One single mutation, F508del, generally associated with severe disease, accounts for about 70% of CF chromosomes world wide, although with a heterogeneous geographical distribution.[5] Patients homozygous for the F508del mutation have the classical severe form of the disease which includes chronic mucous obstruction of the lung and conducting airways, followed by recurrent infections mostly by *Pseudomonas aeruginosa* (Pa) and *Staphylococcus aureus* (Sa), exocrine pancreatic insufficiency (PI), resulting in failure to gain weight and height, and raised levels of Cl^-, sodium, and potassium in exocrine sweat.[5] However, almost 1000 genetic alterations have been detected in the *CFTR* gene (*CFTR* Mutation Database), most presumed to be disease causing mutations. About half of these are amino acid substitutions (missense mutations) and about 20% are splicing mutations. The remainder are nonsense, frameshift (including small deletions and insertions), and a small proportion of promoter mutations.

The relationship between genotype, that is, the mutations in the *CFTR* gene, and the clinical phenotype of CF patients has been difficult to establish, in particular for lung disease.

It was previously shown that the 3272-26A>G mutation leads to the creation of an alternative acceptor splice site competing with the normal one during RNA processing and resulting in the occurrence of an alternatively spliced mRNA with 25 extra nucleotides from intron 17a and a premature stop codon soon thereafter.[6] Previously it was reported that three patients carrying the 3272-26A>G mutation in one of their *CFTR* alleles had mild CF.[7] Another patient with the 3272-26A>G/F508del genotype was reported to have severe CF.[8] Here, we report the clinical phenotypes of 60 CF patients from several European centres, with the 3272-26A>G mutation on one *CFTR* allele, and mostly another severe mutation (73% F508del) on the other allele. We compare them, by statistical methods, with the clinical phenotypes of F508del homozygotes (n=89) from the same centres, matched for age and sex as exactly as possible.

Subjects and methods

PATIENTS

Patients were clinically and genetically characterised in the various CF centres involved in this study (table 1). For the control group, one (two, when possible) F508del homozygous patient for each patient with the 3272-26A>G mutation was selected from the same centres, matching for age and sex with the 3272-26A>G patients as far as possible. However, CF patients with 3272-26A>G were generally among the oldest CF patients in each centre. Therefore, for some cases it was not possible to have two control F508del homozygotes exactly matched. In such cases only one was included and this was chosen as the oldest F508del homozygote from the same centre. The mean current age (SD) of patients in the groups of 3272-26A>G (n=60) and F508del homozygous (n=89) patients were 20.5 (SD 17.5) and 17.0 (SD 11.5) years, respectively. It was found, however, that this difference was not significant (data not shown).

DNA ANALYSIS

Genomic DNA was isolated from peripheral blood lymphocytes according to standard protocols. The 3272-26A>G mutation (as well as non-F508del mutations on the other allele) were detected either by single stranded conformation analysis (SSCA)[9] or by denaturing gradient gel electrophoresis (DGGE)[10] after PCR amplification of genomic DNA in the region of the corresponding *CFTR* exon. Amplicons with abnormal patterns were sequenced either by the ABI PRISM™ Dye Terminator Cycle Sequencing System (Perkin-Elmer, Norwalk, CA) or by the dideoxy manual method with a [^{35}S] nucleotide.

Detection of the F508del mutation was either by dot blotting, by amplification refractory mutation system (ARMS),[11] heteroduplex analysis on polyacrylamide gel electrophoresis (HA-PAGE),[12] or oligonucleotide ligation assay (OLA).[13]

Microsatellites IVS8$(CA)_n$ were investigated as described previously.[14] The IVS8 $(TG)_nT_m$ polymorphic tract was also analysed as previously described[15] and sequenced with the following primer: 5'GAAATTACTGAAGAAGAGGC3'. The GATT tetranucleotide in intron 6a IVS6a-$(GATT)_n$ was analysed by PCR amplification and electrophoresis of the products in a 12% (w/v) polyacrylamide gel.[16] The diallelic markers XV-2c/*Taq*I, KM.19/*Pst*I, MP6-D9/*Msp*I, J44/*Xba*I, M470V/*Hph*I (1540A>G), and T854/*Ava*II (2694G/T) were analysed by PCR amplification and digestion with appropriate restriction enzymes as described previously.[17]

Table 1 Distribution of CF patients in this study according to their genotype and country

	3272-26A>G/any		
Country/No patients	*F508del*	*Other mutation*	*Control group (F508del/F508del)*
France/26	18	8 (P99L; 1717-1A>G; G542X; W846X; R1162X, 2 sibs; N1303K; NI*)	34
Spain/10	6	4 (L206W; 2869insG; R1162X, 2 sibs)	16
Greece/9	7	2 (E822X, 2 sibs)	18
Germany/9	8	1 (W1282X)	13
Portugal/5	5	0	6
Belgium/1	0	1 (4218insT)	2
Total	44	16	89

*NI = not identified.

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Table 2 Clinical features the two groups of CF patients

	Genotype			
	3272-26A>G/any (n=60)		*F508del/F508del (n=89)*	
	Median (QD)	*Mean (SD)*	*Median (QD)*	*Mean (SD)*
Sex (male / female)	35/24 (n=59)		48/41 (n=89)	
Age at diagnosis (y)	12.3 (13.3) (n=55)	8.0 (20.0)	3.8 (6.6) (n=88)	1.0 (4.7)
Sweat test (mEq/l)	99.4 (22.7) (n=42)	99.5 (24.7)	107.3 (19.4) (n=63)	105.0 (24.0)
FEV_1, % predicted	79.7 (27.7) (n=43)	87.0 (47.0)	62.7 (30.2) (n=67)	63.0 (50.0)
FVC, % predicted	93.0 (24.3) (n=42)	88.3 (22.6)	78.5 (37.0) (n=66)	72.5 (26.2)
Lung colonisation with bacterial pathogens*				
Pa (yes/no)	18/34 (n=52)		67/18 (n=85)	
Bc (yes/no)	1/51 (n=52)		5/80 (n=85)	
Hi (yes/no)	7/45 (n=52)		8/77 (n=85)	
Sa (yes/no)	17/35 (n=52)		28/57 (n=85)	
Other (yes/no)	2/50 (n=52)		8/78 (n=85)	
All-Pa (yes/no)	22/30 (n=52)		37/48 (n=85)	
Pancreatic function (PS/PI)	39/16 (n=55)		9/79 (n=88)	
Meconium ileus (yes/no)	0/54 (n=54)		3/83 (n=86)	
Centile - height	50.0 (42.9) (n=22)	46.6 (30.7)	25.0 (40.0) (n=36)	34.5 (29.3)
Centile - weight	50.0 (59.4) (n=22)	47.0 (30.0)	25.0 (40.0) (n=36)	30.9 (26.0)
BMI	22.0 (3.5) (n=25)	21.2 (2.6)	19.0 (4.5) (n=41)	19.6 (2.6)
CN score	13.8 (20.0) (n=19)	7.0 (12.0)	11.9 (9.1) (n=34)	10.0 (14.8)
SK score	81.8 (17.7) (n=34)	90.0 (15.7)	78.6 (16.1) (n=53)	80.0 (18.0)
Nasal polyposis (yes/no)	19/32 (n=51)		6/62 (n=68)	
Other clinical features (yes/no)	22/19 (n=41)		35/30 (n=65)	

*Bc, *Burkholderia cepacia*; Hi, *Haemophilus influenzae*; Pa, *Pseudomonas aeruginosa*; Sa, *Staphylococcus aureus*; All-Pa, all bacterial pathogens except for Pa; BMI, body mass index; CK, Chrispin-Norman score; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; PI, pancreatic insufficient; PS, pancreatic sufficient; SK, Shwachman-Kulczycki score.

CLINICAL PHENOTYPES

The clinical data included in this study were age at diagnosis, sweat test values, pulmonary status assessed by forced expiratory volume in one second (FEV_1) % predicted, and forced vital capacity (FVC) % predicted (which are predicted values for the non-CF population by Knudson *et al*[18]), lung colonisation with bacterial pathogens, pancreatic status, history of meconium ileus, weight and height centiles (for patients under 18) or body mass index (BMI, for patients over 18), Chrispin-Norman (CN) chest radiological score (from 0 to 38, with 0 being the best score, as defined by Conway and Littlewood[19]), Shwachman-Kulczycki (SK) general status score (100 is the best score, also as defined by Conway and Littlewood[19]), nasal polyposis, and other clinical complications or abnormalities.

STATISTICAL ANALYSIS

Data for all clinical parameters are presented (table 2) as the mean (SD) as well as median and interquartile deviation (QD). For distributions of quantitative measurements, the hypothesis of equal variances between the two groups of CF patients under study was tested using Levene's test and there was no evidence to reject it ($p>0.05$), except for current age, age at diagnosis, and weight centile (table 3). Thus, for these two distributions, the Mann-Whitney U test (Wilcoxon) for two independent samples[20] was applied. For the other distributions with equal variances, statistical significance comparisons between the two groups of CF patients were performed using the parametric Student's *t* test for two unpaired samples.[20] For qualitative distributions, the hypothesis of association was tested with each of the two groups of CF patients, considered as two independent samples in 2×2 crosstabs (1 degree of freedom), using both the Pearson chi-square calculation with continuity correction and Fisher's exact test.[20] Coefficients with a p value less than 0.05 were considered to be statistically significant. The SPSS® for Windows software (SPSS Inc, Chicago, IL) was used for all statistical calculations.

Results

We have compared the clinical phenotypes of 60 CF patients with the *CFTR* genotype 3272-26A>G/any mutation with those of 89 patients who were F508del homozygotes, that is, presenting classical CF disease. Patients with the 3272-26A>G mutation were from six different countries, namely France, Spain, Greece, Germany, Portugal, and Belgium (table 1). Among these, 44 (73%) patients were F508del compound heterozygotes (one of them died at the age of 59 years and another one received a lung transplant at the age of 34), four patients were heterozygotes for R1162X (two sib pairs) and two (also sibs) for E822X (table 1). Nine patients carried the following mutations in the other *CFTR* allele (one of each): W1282X, 2869insG, L206W, N1303K, 1717-1 G>A, G542X, 4218insT, W846X, P99L. Except for the P99L and L206W substitutions, all the mutations are known to represent severe CF alleles. For one of the patients carrying the 3272-26A>G mutation the second mutation was not identified (table 1). However, the clinical phenotype was clearly CF, although mild, so the patient was included in the study.

Table 3 Significance tests for comparisons of clinical features between cystic fibrosis patients with 3272-26A>G/any mutation and F508del/F508del genotypes

*Parameter**	*Total No studied*	*Levene's test p value*	*Equal variances*	*Test applied*	*p value*	*Significance*
Age at diagnosis	143	7.2×10^{-9}	No	Mann-Whitney	7.8×10^{-6}	†
Sweat test	105	4.6×10^{-1}	Yes	Student's *t*	5.9×10^{-2}	NS
FEV_1, % predicted	110	5.2×10^{-1}	Yes	Student's *t*	3.8×10^{-3}	†
FVC, % predicted	108	1.5×10^{-1}	Yes	Student's *t*	1.8×10^{-3}	†
Lung colonisation with bacterial pathogens						
Pa	137	—	—	Pearson's χ^2	5.9×10^{-7}	†
				Fisher's exact	5.3×10^{-7}	
Bc	137	—	—	Pearson's χ^2	5.0×10^{-1}	NS
				Fisher's exact	4.1×10^{-1}	
Hi	137	—	—	Pearson's χ^2	6.5×10^{-1}	NS
				Fisher's exact	5.7×10^{-1}	
Sa	137	—	—	Pearson's χ^2	1.0	NS
				Fisher's exact	1.0	
Other	137	—	—	Pearson's χ^2	3.9×10^{-1}	NS
				Fisher's exact	3.2×10^{-1}	
All-Pa	137	—	—	Pearson's χ^2	1.0	NS
				Fisher's exact	1.0	
Pancreatic function	143	—	—	Pearson's χ^2	0	†
				Fisher's exact	5.9×10^{-14}	
Meconium ileus	140	—	—	Pearson's χ^2	4.3×10^{-1}	NS
				Fisher's exact	2.8×10^{-1}	
Centile - height	58	2.9×10^{-2}	Yes	Student's *t*	1.4×10^{-2}	NS
Centile - weight	58	9.3×10^{-1}	No	Mann-Whitney	4.8×10^{-2}	†
BMI	66	3.5×10^{-2}	Yes	Student's *t*	1.5×10^{-6}	†
				Mann-Whitney	2.0×10^{-2}	
CN score	53	1.9×10^{-1}	Yes	Student's *t*	6.3×10^{-1}	NS
SK score	87	4.2×10^{-1}	Yes	Student's *t*	3.9×10^{-1}	NS
Nasal polyposis	119	—	—	Pearson's χ^2	4.0×10^{-4}	†
				Fisher's exact	2.2×10^{-4}	
Other clinical features	106	—	—	Pearson's χ^2	1.0	NS
				Fisher's exact	1.0	

*Abbreviations as in table 2.
†Significant ($p<0.05$), NS = non-significant.

HAPLOTYPE BACKGROUNDS

Most of the patients analysed (80%) had the haplotype D (2,2) at the XV-2c/KM19 loci associated with the 3272-26A>G mutation. For these, an extended haplotype analysis, when done, showed the same variants, being thus consistent with the presence of the same mutant allele in all these patients. The 3272-26A>G mutation was also found in alleles with haplotypes B (1,2) and C (2,1) at XV-2c/KM19 in one French patient and in another previously described Belgian patient,[21] respectively. The other markers in the extended haplotype for the Belgian patient were all the same as for D.

Four other patients, representing about 13% of patients analysed (one in Greece, one in Germany, and two in France) had the haplotype A (1,1) at XV-2c/KM19 in linkage disequilibrium with the 3272-26A>G mutation. An extended haplotype with other markers was determined for the German and the Greek patients, differing substantially from those present in the D allele.

CLINICAL PHENOTYPES

Table 2 shows the average values (mean and median) for the incidence of several clinical parameters of the CF patients in this study. Data are shown for a total of 60 patients with the *CFTR* genotype 3272-26A>G/any mutation and for 89 F508del/F508del patients used as the control group. Differences between these clinical parameters in the two groups of patients were tested for significance (see Methods). Results from the statistical analysis, and reference to the test applied in each case, are shown in table 3. Significant differences were found for the following parameters: age at diagnosis (higher for the 3272-26A>G group), FEV_1 and FVC (also higher), incidence of lung colonisation with Pa (lower), occurrence of pancreatic insufficiency (lower), weight centile or BMI (higher), and nasal polyposis (higher). Altogether these results indicate milder CF disease in patients carrying the 3272-26A>G mutation on one *CFTR* allele. For none of the other parameters (electrolyte concentration in sweat, colonisation with pathogens other than Pa, occurrence of meconium ileus, height centile, and SK and CN scores) were differences found to be significant.

Discussion

Patients who have milder symptoms or atypical CF often have one severe and one so called class V mutation.[22] This class includes mutations that leave residual levels of normal *CFTR* transcripts and protein.[23] Mild CF disease was generally reported for the following class V (splicing) mutations: 3849+10 kb C>T,[24] IVS8-5T,[9 25] and 2789+5G>A.[26] However, the severity of the disease resulting from these mutations was never assessed for significance in comparison to typical CF, that is, presented by most F508del homozygotes. Yet this information is important for genetic counselling, in particular for prenatal diagnosis. Here, we report that patients with the 3272-26A>G mutation (and another *CFTR* mutation, mostly a severe one) exhibit significant differences for various major clinical parameters in comparison to typical CF disease.

TWO INDEPENDENT ORIGINS FOR 3272-26A>G IN EUROPE

Among the 29 patients included in this study whose haplotypes at the XV-2c/KM19 loci were determined and from data on one patient

published elsewhere,[21] 80% have the D haplotype (2,2) in association with the 3272-26A>G mutation.

However, haplotype data also suggest that the 3272-26A>G change must have occurred through a second mutational event. Indeed, four other patients (two French, one German, and one Greek) carry the 3272-26A>G mutation in association with the A haplotype at the XV-2c/KM19 loci. According to data available from the German patient (and also partially from the Greek patient) for other markers, this is a totally different allele, thus strongly suggesting a second origin for the 3272-26A>G mutation. Possibly this happened in populations living in more eastern regions of Europe, considering the origin of two of these patients (one Greek and another from former East Germany). Owing to the lower frequency of allele A (13%) in comparison to D (80%) in association to the 3272-26A>G mutation, the former may have occurred later. Haplotype data suggesting the further change/recombination of the D allele after occurrence of the splicing mutation also support this hypothesis.

The A haplotype is associated with the TG_{11} repeat (*v* TG_{10} for the D haplotype). Since the length of the TG repeat in intron 8 seems to correlate with the extent of exon 9 alternative splicing, that is, the higher the number of TG repeats, the lower the amount of exon 9^+ transcripts produced,[27] it might be expected that the patients with the A haplotype would have a lower amount of exon 9^+ transcripts and hence a more severe phenotype. Although only four of the 3272-26A>G patients analysed here had the A haplotype, no significant difference in clinical phenotype was found for these patients in relation to those with haplotype D.

3272-26A>G CAUSES MILD CF

Patients carrying the 3272-26A>G mutation on one allele (and mostly a severe *CFTR* mutation on the other) were shown here to be diagnosed later, to have better lung function, lower incidence of lung colonisation with Pa, and more often to have normal pancreatic function and higher weight centiles or BMI than patients homozygous for F508del. Altogether, such results indicate milder CF disease in these patients than typical CF. Unexpectedly, nasal polyposis occurs more frequently in these patients (about 37% as opposed to around 10% in F508del homozygotes). It is plausible that nasal polyposis may be underdiagnosed in this study (particularly in the group of F508del homozygotes), since it has been reported to occur generally in about 37% of CF patients[28] and, in particular, in about 40% of F508del homozygotes.[29] The 5T allele, generally associated with mild CF, was also detected with increased frequency in subjects with sinopulmonary disease of ill defined aetiology.[30] Thus, the incidence of nasal polyposis, which is generally considered as a minor complication, does not disprove the fact that patients with 3272-26A>G on one allele have milder CF than F508del homozygotes.

This mutation has been previously shown[6] to create an alternative acceptor splicing site in intron 17a that competes with the normal one but still allows some normal *CFTR* mRNA to be produced. We postulate that the remaining normal *CFTR* mRNA still existing in these patients lessens the severity of CF disease. The molecular basis of this significantly milder CF phenotype must thus lie in the existence of remaining *CFTR* mRNA that is still normally processed, thus giving rise to functional protein.[6] This, however, is not enough to completely avoid lung disease. There are reports of other mutations, both in the *CFTR* gene (reviewed by Kerem and Kerem[31]) and in other genes such as β globin,[32] indicating that reduction in the normal protein levels generally causes milder disease than mutations leading to total absence of or non-functional protein.

The variability and organ involvement in patients carrying this mutation may critically depend on the levels of *CFTR* mRNA (and protein) still present. Differences between the normal and the alternative splicing processes can result from differential expression of splicing factors which will thus act as modifying factors of *CFTR* expression.[33] Indeed, some variability was observed among patients included in this study. Some patients (with F508del on the other allele) were diagnosed at an early age, have Pa colonisation, reduced lung function, and are pancreatic insufficient. Others have very different clinical records. One patient (with F508del on the other allele) was only diagnosed at the age of 32 because of persistent cough during her first pregnancy. Another (also with F508del on the other allele) at the age of 37 had still not developed lung disease, nor pancreatic insufficiency, and was only genotyped because of congenital bilateral absence of the vas deferens (CBAVD).

Owing to the relatively high incidence of this mutation in Europe, it might have been expected that one or more homozygotes for this mutation would be found, as described for other mutations of comparable incidence.[24 34] One possible explanation for our failure to identify any homozygotes is that they may not be CF patients. In light of the fact that just one 3272-26A>G allele, in compound heterozygosity with a severe mutation (like F508del), causes a significant reduction in phenotypic severity, it is plausible that two 3272-26A>G alleles would avoid CF totally.

Further characterisation of *CFTR* splicing in these patients, namely by quantifying normal messenger still present, is our current research, and it will be useful to estimate the minimum levels of *CFTR* necessary to avoid CF lung disease. It is hoped that these insights may also provide clues for novel therapies, through the modulation of factors that enhance the normal versus the alternative splicing.

Electronic database information. Online Mendelian Inheritance in Man (OMIM), http: //www.ncbi.nlm.nih.gov/Omim (for CF MIM 219700). *CFTR* Mutation Database, http: //www.genet.sickkids.on.ca. (for genetic alterations detected in the *CFTR* gene).

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- We report here clinical phenotypes of 60 cystic fibrosis (CF) patients from six European countries with the 3272-26A>G mutation on one allele of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, and another mutation (mostly F508del) on the other allele. These were compared with the clinical phenotypes of F508del homozygous patients (n=89) from the same centres and matched for age and sex as exactly as possible.
- Clinical phenotypes of CF patients with 3272-26A>G were found to be significantly milder ($p<0.05$) than those of F508del homozygotes, namely older age at diagnosis, better pulmonary function, lower incidence of colonisation with *Pseudomonas aeruginosa*, lower occurrence of pancreatic insufficiency, and higher weight centile or body mass index. Altogether, this information is important for genetic counselling, in particular for prenatal diagnosis.
- Unexpectedly, incidence of nasal polyposis was found to be significantly higher in 3272-26A>G patients.
- The heterogeneity of *CFTR* haplotypes indicates that this mutation, which is spread all over Europe, must have evolved from more than one mutational event. It is shown for the first time that CF patients with one mutation causing alternative splicing (and mostly another severe mutation) have significantly milder disease than patients with two severe mutations. We postulate that the remaining normal *CFTR* mRNA still existing in these patients alleviates the severity of CF disease.

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Maternal gene effect in neurofibromatosis 2: fact or artefact?

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EDITOR—Neurofibromatosis 2 (NF2) is a rare autosomal dominant disease that is characterised by benign nervous system tumours, skin lesions, and ocular abnormalities.[1–3] Two studies have found that NF2 patients with a family history of the disease and with maternal inheritance have more severe disease than inherited cases with paternal inheritance. Kanter *et al*[4] noted that patients with maternal inheritance had an earlier age at onset and Evans *et al*[5] found that patients with maternal inheritance had both an earlier age at onset and an earlier age at death. In both studies, the mean age at onset was 18 years with maternal inheritance and 24 years with paternal inheritance.

These results require confirmation for several reasons. First, Parry *et al*[6] found identical mean ages at onset (22.8 years) in symptomatic NF2 patients with paternal or maternal inheritance. Second, these studies were based on relatively small numbers of patients. Kanter *et al*[4] studied 38 inherited cases, Evans *et al*[5] studied 56 inherited cases, and Parry *et al*[6] studied 36 inherited cases; 66% of the patients in Kanter *et al*[4] and 64% in Evans *et al*,[5] but only 39% in Parry *et al*,[6] had maternal inheritance. Third, in examining the effect of maternal inheritance on disease severity, these studies reported only age at onset (and age at death in Evans *et al*[5]) as indices of disease severity. In addition to age at onset or age at diagnosis, NF2 disease severity is defined by the number of non-vestibular schwannoma cerebral tumours and of spinal tumours.[6] Fourth, none of the studies examined potential confounding factors (such as type of treatment centre and constitutional *NF2* mutation type) that can affect age at onset, age at diagnosis, or mortality. NF2 patients who are treated at non-specialty centres have higher odds of death than those who are treated at specialty centres, and NF2 patients with missense mutations have lower odds of death than those with nonsense or frameshift mutations.[7] Genotype-phenotype correlation studies have found that NF2 patients with constitutional *NF2* missense mutations or large deletions generally have mild disease, those with splice site mutations have variable disease severity, and those with nonsense or frameshift mutations have severe disease.[8–11]

We reassessed the question of maternal gene effect in NF2 with a larger number of patients and with consideration of potential confounders. We used data from the United Kingdom NF2 Registry, based in the Department of Medical Genetics, St Mary's Hospital, Manchester. Patients are ascertained by contacting neurosurgeons, neurologists, otolaryngologists, paediatricians, dermatologists, and geneticists throughout the United Kingdom, augmented in the North West Region by the Regional Cancer Registry. As of 15 September 2000, the registry had data on 140 inherited cases (85 with maternal inheritance and 55 with paternal inheritance, including the 56 inherited cases previously reported by Evans *et al*[5]). All patients met the Manchester clinical diagnostic criteria for NF2[5] or had identified constitutional *NF2* mutations.

For univariate analyses, the two tailed *t* test was used for age variables, the χ^2 test for discrete variables (for example, distribution of mutation types), and life tables for mortality analysis. Multivariate analyses were used to examine the independent effects of covariates (linear regression for age variables and the Cox proportional hazards model for mortality analysis). Because age at onset and age at diagnosis are highly correlated (in the present study, $r^2 = 0.78$, $p<0.001$), separate multiple linear regressions were done with each age variable as the outcome. p values ≤ 0.05 were considered to be statistically significant.

In univariate comparisons of patients with maternal and paternal inheritance, patients with maternal inheritance were treated slightly more frequently at non-specialty centres (75% versus 62%, p=0.10) and had a slightly lower mean age at onset (23.1 years versus 25.5 years, p=0.30). The distribution of constitutional *NF2* mutation types varied significantly

Table 1 Results of multiple linear regressions

	Outcome			
	Age at onset (y)		*Age at diagnosis (y)*	
Covariate	*b (95% CI)*	*p*	*b (95% CI)*	*p*
Type of treatment centre (specialty compared to non-specialty)	−4.7 (−9.8,0.4)	0.07	−10.5 (−16.0,−5.0)	<0.001
Constitutional *NF2* mutation type (compared to nonsense or frameshift)				
Splice site	0.1 (−6.8,7.0)	0.99	2.4 (−5.4,10.2)	0.54
Missense	17.2 (9.6,24.8)	<0.001	24.1 (15.3,32.9)	<0.001
Large deletions	7.1 (0.5,13.6)	0.04	10.8 (3.4,18.2)	0.01
Unidentified	5.3 (−1.6,12.2)	0.13	6.4 (−1.6,14.4)	0.12

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