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A Single-Gene Explanation for the Probability of Having Idiopathic Talipes Equinovarus

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Summary

It has been hypothesized that the pathogenesis of idiopathic talipes equinovarus (ITEV, or clubfoot) is explained by genetic regulation of development and growth. The objective of the present study was to determine whether a single Mendelian gene explains the probability of having ITEV in a sample of 143 Caucasian pedigrees from Iowa. These pedigrees were ascertained through probands with ITEV. Complex segregation analyses were undertaken using a regressive logistic model. The results of these analyses strongly rejected the hypotheses that the probability of having ITEV in these pedigrees was explained by a non-Mendelian pattern of transmission with residual sibling correlation, a nontransmitted (environmental) factor with residual sibling correlation, or residual sibling correlation alone. These results were consistent with the hypothesis that the probability of having ITEV was explained by the Mendelian segregation of a single gene with two alleles plus the effects of some unmeasured factor(s) shared among siblings. The segregation of alleles at this single Mendelian gene indicated that the disease allele A was incompletely dominant to the nondisease allele B. The disease allele A, associated with ITEV affection, was estimated to occur in the population of inference with a frequency of .007. After adjusting for sex-specific population incidences of ITEV, the conditional probability (penetrance) of ITEV affection given the AA, AB, and BB genotypes was computed to be 1.0, .039, and .0006, respectively. Individual pedigrees in this sample that most strongly supported the single Mendelian gene hypothesis were identified. These pedigrees are candidates for genetic linkage analyses or DNA association studies.

Introduction

Talipes equinovarus is a common birth defect occurring in 1–3/1,000 live births in Caucasians (Cartlidge 1984). This disorder is characterized by a rigid hindfoot equinous, hindfoot varus, midfoot adductus, and cavus deformity. A minority of cases are associated with neuromuscular diseases, chromosomal abnormalities, Mendelian and non-Mendelian syndromes, and, rarely, with externally caused disruptions. The great majority of cases are labeled idiopathic talipes equinovarus (ITEV). These deformities occur in otherwise normal infants.

A genetic etiology for ITEV has been proposed from patterns of ITEV aggregation in families (Carter 1965). A number of studies support the hypothesis that a single Mendelian gene (SMG) explains the probability of having ITEV in pedigrees (Palmer 1964; Chung et al. 1969; Palmer et al. 1974). Yang et al. (1987) reported that the etiology of congenital clubfoot could be attributed to the segregation of an SMG plus polygenes in a multiracial sample ascertained from multiple sources in Hawaii. However, the mode of segregation of this gene could not be determined by those analyses. The SMG hypothesis was supported in two racial subgroups from this sample (Hawaiians and Caucasians), although the segregation parameters in those racial subgroups could not be estimated. This segregation could not be demonstrated in a subgroup of Orientals. Wang

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et al. (1988) have analyzed a sample of Caucasian pedigrees ascertained through probands with ITEV from an Indiana clinic population. Those authors concluded that liability to ITEV could be explained by the autosomal dominant segregation of an SMG with incomplete penetrance, as well as other polygenic factors.

The present study tests the hypothesis that the probability of having ITEV in pedigrees ascertained through probands with ITEV can be explained by allelic variation in an SMG and by sibling correlations. The results presented here support the hypothesis that an SMG and sibling correlation explain the probability of having ITEV. The results do not support the hypothesis of non-Mendelian explanations for the probability of having ITEV.

Demonstration of a significant Mendelian component provides a stimulus to use candidate gene and/or positional cloning strategies to identify one or more genes involved in the etiology of ITEV. This approach has proved successful in other genetically complex disorders, such as cleft lip and palate (Ardinger et al. 1989) and hypertension (Jeunemaitre et al. 1992).

Subjects, Material, and Methods

Sample and Diagnostic Criteria

The present sample was ascertained through 190 Caucasian probands with ITEV presenting to the orthopedic clinic at the University of Iowa Hospitals and Clinics between 1984 and 1989. The affection status of all probands was made by physical examination. All probands had the ITEV deformity characterized by a rigid (nonpassively correctable) equinous of the ankle, varus of the subtalar joint, adductus of the midfoot, and cavus caused by a relative pronation of the forefoot in relation to the hindfoot. No other physical abnormalities were apparent in these probands. No individuals with neuromuscular or syndromic clubfoot were included as probands. Individuals with postural equinovarus feet were excluded as probands. These deformities are passively correctable, tend to spontaneously correct, do not have the characteristic calf atrophy of ITEV, and show no tendency to recur if treated by casts. Of the 190 probands that met the qualifications described here, 147 (77.4%) pedigrees agreed to participate in this study. Four of these pedigrees were excluded from the analysis because reliable family relationship information could not be obtained. The remaining 143 pedigrees comprised the sample used in the present analyses.

The affection status of all first- and second-degree relatives of the proband was determined by medical records and by telephone interview. Of the 24 affected relatives of probands, 8 were evaluated at the University of Iowa. The affection status of these eight was determined directly from medical records. Because no other congenital abnormality displays the deformity that characterizes ITEV and has the treatment pattern of ITEV in an otherwise normal child, affection status of the remainder of affected relatives was based on the presence of the typical deformity at birth and by treatment regimen. The affected individual and/or the parents were asked about the appearance of the foot at birth. Specifically, they were asked whether the "top" of the foot was turned so that it was positioned where the "sole" of the foot should have been. Relatives of probands with feet that were only twisted into an in-toeing position were judged to have metatarsus adductus and were not counted as ITEV cases. Knowledge about the treatment regimen in relatives was used to eliminate mild or postural deformities. If treatment required fewer than five casts or braces alone, the proband's relative was considered not to have ITEV. Relatives with any other congenital abnormality were also considered not to have ITEV. The criteria used to determine affection status in relatives of probands may be considered conservative, in that a few mild cases of ITEV corrected with only a few casts may have been considered as unaffected in the present analysis.

The 143 pedigrees obtained in this manner consisted of 2,436 individuals. Of these, 1,245 (51.1%) were male and 1,191 (48.9%) were female. The pedigrees in this sample ranged in size from 9 to 46 individuals, with an average pedigree size of 17.0 individuals.

Maximum-Likelihood Model and Parameter Estimation

A regressive logistic model of segregation analysis for a dichotomous trait (Bonney 1986) was used to determine whether the probability of having ITEV could be explained by the segregation of an SMG, a nontransmitted (environmental) factor, a single factor segregating in a non-Mendelian manner, residual sibling correlation, or a combination of these etiologies. The regressive logistic model explained the dependence of ITEV status among family members by specifying the logarithm of the odds (logit) of being affected versus unaffected as a function of a single unmeasured factor.

The single unmeasured factor had two possible discrete values, A and B. These combined to form three classes of ousiotypes (Cannings et al. 1978), denoted "AA," "AB," or "BB." The relative frequency of each ousiotype class was in binary proportions. These proportions were described by the single parameter P, such that $\Pr(AA) = P^2$, $\Pr(AB) = 2P(1-P)$, and $\Pr(BB)$

$= (1-P)^2$. For Mendelian models, these proportions corresponded to Hardy-Weinberg genotype frequencies. When no epistasis was assumed, the relationship of these three ousiotype classes to the probability of having ITEV was measured through the logistic regressive model parameter β_i, where i = AA, AB, or BB. The probabilities that a parent with ousiotype AA, AB, or BB transmitted an A factor to offspring were denoted by the transmission parameters τ_1, τ_2, and τ_3, respectively.

Residual familial aggregation not explained by the major factor was modeled by assessing correlation of affection status between siblings. These correlations were specified by the covariate Z_{OS1}, defined as the number of affected older siblings, and Z_{OS2}, defined as the number of unaffected older siblings. Sex-specific differences were accounted for in the model by a binary sex covariate denoted X_{sex} (1 = male, 0 = female). The logistic regressive model was therefore specified to be $\log_e[\Pr(\text{ITEV})/\Pr(\text{No ITEV})] = \beta_i + \beta_{sex}X_{sex} + \Sigma_j\gamma_jZ_j$, where β_{sex} measured sex effects, and γ_j (j = OS1, OS2) measured the effects of sibling covariates Z_j. In order to correct for ascertainment through probands, the model likelihood was conditioned on the ITEV status of each proband. Model likelihoods and parameter estimates were obtained using the REGD program of the Statistical Analysis for Genetic Epidemiology (SAGE) package (version 2.0, Sorant and Bonney 1989), in conjunction with the maximization algorithms of MAXFUN (Sorant and Elston 1989).

In order for the parameter estimates obtained from the present segregation analyses to more accurately reflect the observed population prevalence rates of ITEV, additional unrelated individuals were added to the sample of pedigrees (Hecht et al. 1991). These rates have been reported by Palmer et al. (1974) to be 1.62/1,000 males and 0.80/1,000 females in a midwestern U.S. population. To reflect these population rates, 14,907 unrelated individuals were added to the sample. Of these, 12 of 7,407 unrelated males and 6 of 7,500 unrelated females were coded as affected with ITEV.

Hypothesis Testing

Tests of hypotheses were conducted in four sequential steps. First, tests of transmission parameters were made using the most complete ousiotype and residual sibling correlation parameterizations. Second, by using the most parsimonious transmission function(s), tests were made to determine the effects of a major factor on the probability of having ITEV by reducing the parameters of the penetrance parameterization. Third, given the most parsimonious, best-fitting transmission and penetrance parameterization(s), tests of residual family correlations were made. Fourth, given the most parsimonious, best-fitting transmission, penetrance, and sibling correlation parameterization(s), a test of sex effects was made. At each step in the hypothesis testing strategy, models specified by a particular null hypothesis were tested against more complete models that fit as well as the general transmission (GT) model and against the GT model itself.

First, hypotheses were tested to determine the optimal transmission parameterization. These models specified unrestricted effects of genotype, sibling correlation, and sex. The most general transmission model allowed the transmission parameters τ_1, τ_2, and τ_3 to maximize independently between zero and one. This model was compared with three reduced-transmission parameterizations. A nontransmitted (environmental) factor (NTF) model was specified by constraining $\tau_1 = \tau_2 = \tau_3$. Allelic variation at an SMG with large effects on the probability of having ITEV was modeled by constraining the transmission parameters to be $\tau_1 = 1.0$, $\tau_2 = .5$, and $\tau_3 = .0$, as defined under Mendelian inheritance. The hypothesis of no major factor (NMF) was parameterized by constraining the effects of all ousiotypes to be equal (i.e., $\beta_{AA} = \beta_{AB} = \beta_{BB}$), and by constraining the parameter P to have a value of 1. This eliminated from the model the effects of an unmeasured single factor on logit probability of having ITEV.

Second, by using the optimal transmission parameterization(s) determined from the preceding analyses, tests of penetrance parameters were made. These tests were accomplished by placing restrictions on the phenotypic effects of the unmeasured single factor. For SMG models, these restrictions corresponded to dominant, additive, or recessive genetic effects. Under a recessive genetic model, the null hypothesis specified $\beta_{AA} \neq \beta_{AB} = \beta_{BB}$. Under a dominant genetic model, the null hypothesis specified $\beta_{AA} = \beta_{AB} \neq \beta_{BB}$. Under an additive (codominant) genetic model, the null hypothesis specified $\beta_{AB} = 0.5(\beta_{AA} + \beta_{BB})$.

Third, using the optimal transmission and penetrance parameterization(s) determined in the preceding analyses, tests were made to determine the effect of residual sibling correlation on the logit probability of having ITEV. Three null hypotheses were tested by constraining specific γ parameters to be zero. These null hypotheses specified (1) $\gamma_{OS1} = 0$, (2) $\gamma_{OS2} = 0$, and (3) $\gamma_{OS1} = \gamma_{OS2} = 0$.

Fourth, by using the optimal transmission, penetrance, and sibling correlation parameterization(s) determined from the preceding analyses, hypothesis tests were made to determine the effect of sex on the logit

Table 1

Description of Affection Status, by Sex, for the Total Sample

Sex	Affected	Unaffected	Total
Male	107 (4.4%)	1,138 (46.7%)	1,245 (51.1%)
Female	60 (2.5%)	1,131 (46.4%)	1,191 (48.9%)
Total	167 (6.9%)	2,269 (93.1%)	2,436 (100%)

probability of having ITEV. This null hypothesis specified $\beta_{sex} = 0$.

Finally, there was concern that the exclusion of parent-offspring correlation terms may have induced bias in the results of our analysis. Because the data contained very few affected parent-offspring pairs, models containing parent-offspring correlation terms may have been overparameterized with respect to these data. Therefore, hypothesis tests were made comparing models with and without binary parental covariates. These covariates were coded as dummy variables specifying whether an individual's mother or father was affected with ITEV. A pair of covariates specific to mothers and a pair specific to fathers were considered to account in part for the gender differences in ITEV affection. Each model considered in the previous hypothesis testing steps was fitted with and without these parental covariates.

Likelihood ratio statistics were used to test all hypotheses. These statistics compared the likelihood of one model containing a set of estimated parameters with the likelihood of a second model in which a subset of those parameters was constrained to have particular values under a null hypothesis. The likelihood ratio test statistic was computed as minus two times the natural logarithm of the ratio of these two likelihoods. This statistic was distributed as a χ^2 with df equal to the number of parameters constrained under the null hypothesis. In order to compare two models, one of which was not parameterized to be a nested subset of the other, the Akaike information criterion (AIC; Akaike 1974) was defined as twice the likelihood of the model plus twice the number of unrestricted parameters in the model. For any two prespecified models, the model with the smaller AIC was judged to provide a better fit to the data.

Pedigree Identification

To identify those pedigrees that most strongly support one of multiple competing hypotheses of interest, likelihood ratio comparisons were made for each individual pedigree. The competing hypotheses of interest were those identified from the complex segregation analysis described above. The likelihood for one model with p unrestricted parameters (L_p) was compared with the likelihood of a second model in which n of these parameters were restricted under a null hypothesis (L_{p-n}). This comparison was made by computing the statistic $R = -2 \log_e(L_{p-n}/L_p)$ by using the parameter estimates obtained from the total sample for each pedigree. A pedigree was judged to support the hypothesis specified by the more reduced model if $R > 0$, to support the hypothesis specified by the more complete model if $R < 0$, and to support neither hypothesis if $R = 0$.

Results

Affection Status

As presented in table 1, 167 (6.9%) of the individuals in the present sample were affected with ITEV. There were 107 (4.4%) affected males and 60 (2.5%) affected females, with a male:female sex ratio of 1.8:1. The proportion of affected males in the total sample was significantly higher than the proportion of affected females ($\chi^2 = 12.058$, df = 1, $p < .001$). There were 90 male probands and 53 female probands, with a male:female sex ratio of 1.7:1. There were also 17 nonproband affected males and 7 nonproband affected females, with a male:female sex ratio of 2.4:1.

The number of affected individuals per pedigree ranged from one to four (table 2). Twenty (14%) pedigrees had multiple affected individuals. Of the 17 pedigrees with two affected members, 9 (45%) were parent-offspring or sib-sib pairs (i.e., first-degree relationship to the proband), and 8 (40%) were avuncular pairs (i.e., second-degree relationship to proband). Three (15%) pedigrees had more than two affected members. In the first pedigree, the brother, mother, and a paternal uncle of the male proband were affected. In the second pedigree, two paternal uncles of the female proband were

Table 2

Distribution of Affected Individuals across Pedigrees

No. Affected/ Pedigree	No. of Pedigrees	Mean No./ Pedigree	Pedigree Size Range
1	123	16.77	9–46
2	17	19.94	11–33
3	2	17.50	17–18
4	1	14.00	14
Total	143	17.03	9–46

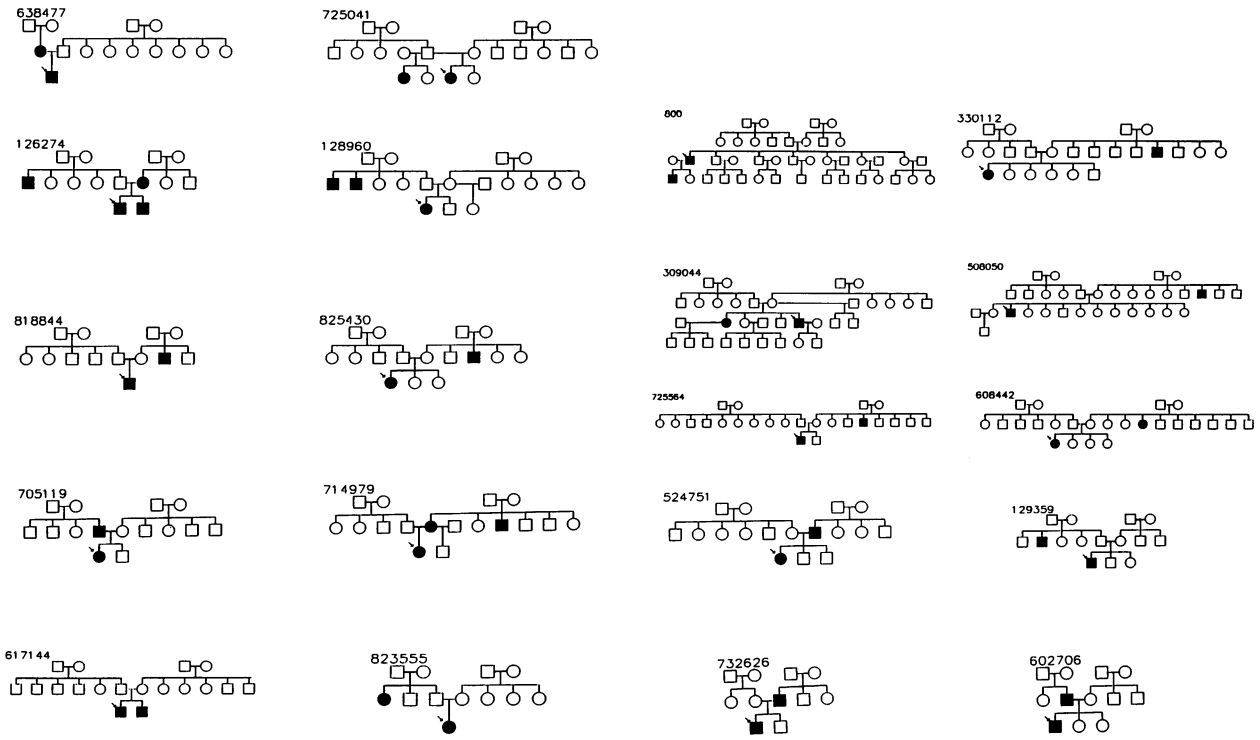

Figure 1 Pedigrees with multiple ITEV cases. Arrows indicate probands through which the pedigree was ascertained.

affected. In the third pedigree, the mother and maternal uncle of the female proband were affected. Drawings of the 20 multiply affected pedigrees are presented in figure 1. The ratio of affected males:females in the multicase families was 1.7:1.

Genetic Analysis

The maximization algorithms implemented in SAGE allow values of the β and γ parameters in a segregation analysis to be estimated outside interpretable limits (e.g., <-10 or >10 on the logit scale). For practical purposes, the conditional probability, r, of having ITEV, given ousiotype i, X_{sex}, or Z_j, is essentially zero when β and γ are <-10 and is essentially one when β and γ are >10. Exploratory analyses (authors' unpublished results) indicated that these point estimates varied widely without accompanying changes in the model likelihood. Therefore, model likelihoods and parameter estimates were recomputed after β and γ parameters with large positive or negative estimates were fixed at one or more of the values of logit (r) or logit ($1-r$) specified in table 3. These values represent order or magnitude changes in the logit probability of having ITEV. For example, if the logistic regression coefficient associated with the AA ousiotype was estimated to be 18.42 on the logit scale, then the corresponding probability of having ITEV, given AA ousiotype, is .99999999. To test the range of values over which the model likelihood did not change, the logit scale parameter β_{AA} was fixed at at 11.51, 13.82, and 16.12, and the remaining model parameters were reestimated.

Table 4 presents the maximum-likelihood parameter estimates and hypothesis test results for four different models of transmission with the most complete penetrance and sibling correlation parameterizations. The NTF and NMF models did not fit the data as well as the GT model did. Therefore, no further model fitting was undertaken for either the NTF or the NMF mod-

Table 3

Values of the Conditional (Logit) Probability (r) of Having ITEV

r	logit(r)	logit($1-r$)
1×10^{-8}	−18.42	18.42
1×10^{-7}	−16.12	16.12
1×10^{-6}	−13.82	13.82
1×10^{-5}	−11.51	11.51

Table 4

Hypothesis Tests of Best Transmission Parameterization, Given Sibling Correlation, with Adjustment for Population Prevalence by Addition of 14,907 Unrelated Individuals

Parameter	GT Model[a]	NTF Model[b]	SMG Model[b,c]	NMF Model[b]
P	.008	.023	.009 ± .004	(1)
β_{AA}	11.315	8.136	10.543 ± 38.780	−6.556
β_{AB}	−3.543	−4.317	−3.610 ± .516	(β_{AA})
β_{BB}	−7.390	{<−18.42}[d]	−7.461 ± .388	(β_{AA})
γ_{OS1}	−4.332	−3.373	−4.204 ± 1.218	−1.423
γ_{OS2}	1.113	1.935	1.017 ± .118	.785
β_{sex}	.530	.935	.644 ± .541	2.040
τ_1	[1]	.077	(1)	...
τ_2	.527	(τ_1)	(0.5)	...
τ_3	[0]	(τ_1)	(0)	...
$-2 \log_e L$	478.895	489.838	483.456	511.455
AIC	498.895	505.838	497.456	519.455
χ^2	...	10.943	4.561	32.560
df	...	2	3	6
p value	...	.004	.207	<.001

[a] Square brackets indicate that parameter was maximized at the indicated boundary value.
[b] Parentheses indicate that parameter was constrained to have the indicated value.
[c] ± Standard error estimates for models fitting as well as the GT model.
[d] Boundary value.

els. The SMG model did fit the data as well as the GT model did ($\chi^2 = 1.741$, df = 3, $p = .628$). These results provided support for the hypothesis that the segregation of an SMG explained the probability of having ITEV in this sample and rejected the alternative non-Mendelian explanations. The subsequent analyses were undertaken to more precisely describe the nature of this segregating gene.

Table 5 presents maximum-likelihood parameter estimates and hypothesis test results for SMG models with reduced penetrance functions. Genetic models that specified dominant, recessive, or additive genotype effects (table 5) did not fit the data as well as did the more complete SMG and GT models with unrestricted genotype effects (table 4). Therefore, a model that described the segregation of an SMG with unrestricted genotype effects was judged to most parsimoniously explain the probability of having ITEV in this sample of pedigrees.

Table 6 presents maximum-likelihood parameter estimates and hypothesis test results for SMG models with unrestricted genotype effects that specified reduced residual sibling correlation parameterizations. All models with reduced sibling correlation parameterizations (models 1–3 in table 6) fit the data significantly worse than did the most complete model with unrestricted genotype effects and the complete sibling correlation parameterization (table 4). Therefore, the probability of having ITEV was explained by both an SMG and residual correlation among siblings.

Table 6 also presents maximum-likelihood estimates for the SMG model with unrestricted genotype effects, sibling correlation, and no sex effects. A model without sex effects (model 4 in table 6) fit the data as well as did the GT and SMG models with unrestricted ousiotype or genotype effects, unrestricted sibling correlation, and unrestricted sex effects (table 4). Therefore, the SMG effects are the same in both genders after adjusting for population ITEV prevalence.

Parameter estimates for the best fitting, most parsimonious model (model 4) are presented in table 6. The frequency of the allele associated with the probability of having ITEV was estimated to be .007 ± .003 in this sample. The regression coefficients for an SMG model with unrestricted phenotypic effects were estimated on the logit scale to be bounded at 11.51 for the AA genotype, −3.215 ± .329 for the AB genotype, and −7.511 ± .393 for the BB genotype. Estimates of shared sibling effects were −4.465 ± 1.190, associated with Z_{OS1}, and 1.046 ± .118, associated with Z_{OS2}.

The results of model fitting suggested instability in the numerical results in the β_{AA} parameter. We observed large standard errors associated with the estimate of β_{AA} in models containing a single major factor.

Table 5

Hypothesis Tests of the Best Penetrance Function Parameterization, Given Mendelian Transmission

Parameter	Dominant	Additive	Recessive
P	.004	.017	.024
β_{AA}	−3.062	−.073	7.967
β_{AB}	(β_{AA})	(.5(β_{AA} + β_{BB}))	−7.345
β_{BB}	−7.064	−7.986	(β_{AB})
γ_{OS1}	−1.867	−3.014	−10.353
γ_{OS2}	.914	1.021	.927
β_{sex}	1.135	.811	2.427
$-2 \log_e L$	496.499	490.770	489.416
AIC	508.499	502.770	501.416
$\chi^{2\,a}$	13.043	7.314	5.960
p value	−.001	.007	.015
$\chi^{2\,b}$	17.604	11.875	10.521
p value	.003	.037	.062

NOTE.—Parentheses indicate that parameter was constrained to have the indicated value.

[a] 1-df comparison with SMG model; see table 3.

[b] 5-df comparison with GT model; see table 3.

These estimates and standard errors were consistently retrieved after consideration of multiple starting values and maximization conditions. When the β_{AA} parameter was fixed at various values within a 95% confidence interval of a β_{AA} point estimate, there was no change in the likelihood of the model in question. Therefore, within the constraints of the algorithms of MAXFUN, the point estimates of β_{AA} presented here can be considered convergent maximum-likelihood solutions.

We also observed that the estimate of γ_{OS1} was not different from zero in the absence of a single major factor (e.g., NMF model in table 4). This estimate became significantly negative in the presence of a single major factor (e.g., SMG model in table 4). We also observed that the estimate of β_{AA} was substantially smaller when γ_{OS1} was forced to be zero (e.g., models 1 and 3 in table 6) than when γ_{OS1} was estimated (e.g., models 2 and 4 in table 6). These observations may be the result of simultaneously considering a single major factor, sibling correlation, and adjustment for population rates of ITEV. For models that included the effects of a single major factor, negative estimates of γ_{OS1} suggested a deficiency of affected older siblings in the present sample, in comparison with expected population prevalence rates.

Models were also fitted that simultaneously considered parental covariates and the other parameters shown in tables 4–6. χ^2 statistics comparing models with and without parental covariates are presented in table 7. The inference of a single major gene was the same with (table 7) and without (tables 4–6) parental

Table 6

Hypothesis Tests of Sibling Correlation and Sex Effects for Mendelian Transmission Models with Arbitrary Genotype Effects

Parameter	Model 1	Model 2	Model 3	Model 4[a]
P	.014	.007	.020	.007 ± .003
β_{AA}	.541	{>16.12}[b]	−1.149	{>11.51}[b]
β_{AB}	−4.201	−3.329	−3.969	−3.215 ± .329
β_{BB}	−7.356	−7.300	−7.924	−7.511 ± .393
γ_{OS1}	(0)	−11.095	(0)	−4.465 ± 1.192
γ_{OS2}	.837	(0)	(0)	1.046 ± .118
β_{sex}	1.077	.770	.930	(0)
$-2 \log_e L$	499.785	521.860	530.434	484.957
AIC	511.785	533.860	540.434	496.957
$\chi^{2\,c}$	16.329	38.404	46.978	1.501
df	1	1	2	1
p value	<.001	<.001	<.001	.221
$\chi^{2\,d}$	20.890	42.965	51.539	6.062
df	4	4	5	4
p value	<.001	<.001	<.001	.195

NOTE.—Parentheses indicate that parameter was constrained to have the indicated value.

[a] ± Standard error estimates for models fitting as well as SMG model; see table 3.

[b] Boundary values.

[c] Compared with SMG model; see table 3.

[d] Compared with GT model; see table 3.

Table 7

Hypothesis Tests of Models Depicted in Tables 4–6, Containing Parental Covariates

Model	−2 ln Likelihood			
	With Parents	Without Parents	χ^2 (df)[a]	χ^2 (df = 4)[b]
GT[c]	470.849	478.895	...	8.046
NTF[c]	480.135	489.838	9.286* (2)	9.703*
SMG[c]	476.429	483.456	5.580 (3)	7.027
NMF[c]	499.074	511.455	28.225* (6)	12.381*
Dominant[d]	493.312	496.499	22.463* (4)	3.187
Additive[d]	482.672	490.772	11.823* (5)	8.100
Recessive[d]	483.450	489.416	12.601* (4)	5.966
Model 1[e]	499.363	499.785	28.514* (4)	.422
Model 2[e]	521.397	521.860	50.548* (4)	.463
Model 3[e]	527.685	530.434	56.836* (5)	2.749
Model 4[e]	478.128	484.957	7.279 (4)	6.829

[a] Likelihood ratio χ^2 comparison of models containing parental covariates with the GT model containing parental covariates.

[b] Likelihood ratio χ^2 comparison of corresponding models with and without parental covariates.

[c] See table 4.

[d] See table 5.

[e] See table 6.

* $p < .05$.

covariates. Parental covariates did not contribute significantly to any model that included a single major gene but did contribute to the NTF and NMF models. For models not rejected when compared with the GT model, the point estimates of the remaining model parameters did not differ substantially between models with and without parental covariates (results not shown), as determined by computing 95% confidence intervals about specific parameter estimates in models with and without parental covariates.

Pedigree Identification

Likelihood ratio comparisons were made to identify individual pedigrees that supported the SMG hypothesis. These comparisons contrasted the SMG hypothesis to two competing hypotheses of interest. The competing hypotheses specified either sibling correlation with NMF (i.e., the NMF model) or a major factor with GT (i.e., non-Mendelian) parameters plus sibling correlation (i.e., the GT model). None of these models considered sex differences. These contrasts provide an exploratory means of identifying specific pedigrees that contribute to deviations in parameter estimates of interest. For example, the contrast between the SMG and GT models identifies pedigrees in which Mendelian transmission is most likely.

The contrasts were made by using two likelihood-ratio statistics. The statistic *R1* was defined to be $-2 \log_e(L_{SMG}-L_{NMF})$, and the statistic *R2* was defined to be $-2 \log_e(L_{SMG}-L_{GT})$. Individual pedigree likelihoods were obtained from models containing maximum-likelihood parameter estimates obtained from the total sample. First, L_{SMG} was the the likelihood of model 4 in table 6. Second, L_{GT} was the likelihood of a GT model with maximum-likelihood parameter estimates $P = .006$, $\beta_{AA} = 12.636$, $\beta_{AB} = -3.215$, $\beta_{BB} = -7.360$, $\gamma_{OS1} = -4.564$, $\gamma_{OS2} = 1.136$, $\tau_1 = 1.0$, $\tau_2 = .550$, and $\tau_3 = 0$. Third, L_{NMF} was the likelihood of an NMF model with maximum-likelihood parameter estimates $\beta_{AA} = \beta_{AB} = \beta_{BB} = -6.174$, $\gamma_{OS1} = -.874$, and $\gamma_{OS2} = .863$. Values of *R1* or *R2* greater than zero implied support of the SMG model over the NMF or GT models, respectively.

The bivariate distribution of *R1* and *R2* is presented in figure 2. All pedigrees with only a single affected individual had values of *R1* and *R2* near zero. This indicated that pedigrees with a single affected individual provided no substantial support for any of the three models (SMG, NMF, or GT) of interest. Seven pedigrees with multiple affected individuals that fell into quadrant B supported the SMG model over both the NMF and GT models. These pedigrees provided the strongest support for the Mendelian transmission of a single gene. Eleven pedigrees with multiple affected individuals that fell into quadrant D also provided support for the transmission of a single gene, since the transmission parameters of the GT model did not differ significantly from Mendelian expectations. Two pedigrees with multiply affected individuals fell into quadrant C. One of these pedigrees (608442 in fig. 1) fell very close to the point $R1 = R2 = 0$, giving equivocal support for any one of the three models of interest. The other pedigree (508050 in fig. 1) did not favor the segregation of an SMG over the alternative hypotheses specified by the GT and NMF models, with values of *R1* $= -0.9$ and *R2* $= -2.1$. In this pedigree, the proband was one of 12 otherwise unaffected children. The other affected individual in this pedigree was one of nine maternal aunts and uncles. The small proportion of affected individuals in these large sibships apparently provided evidence against the transmission of an SMG in this family. The results presented in figure 2 indicate that 18 of 20 pedigrees with multiple affected individuals provided support for the SMG model over the GT or NMF models.

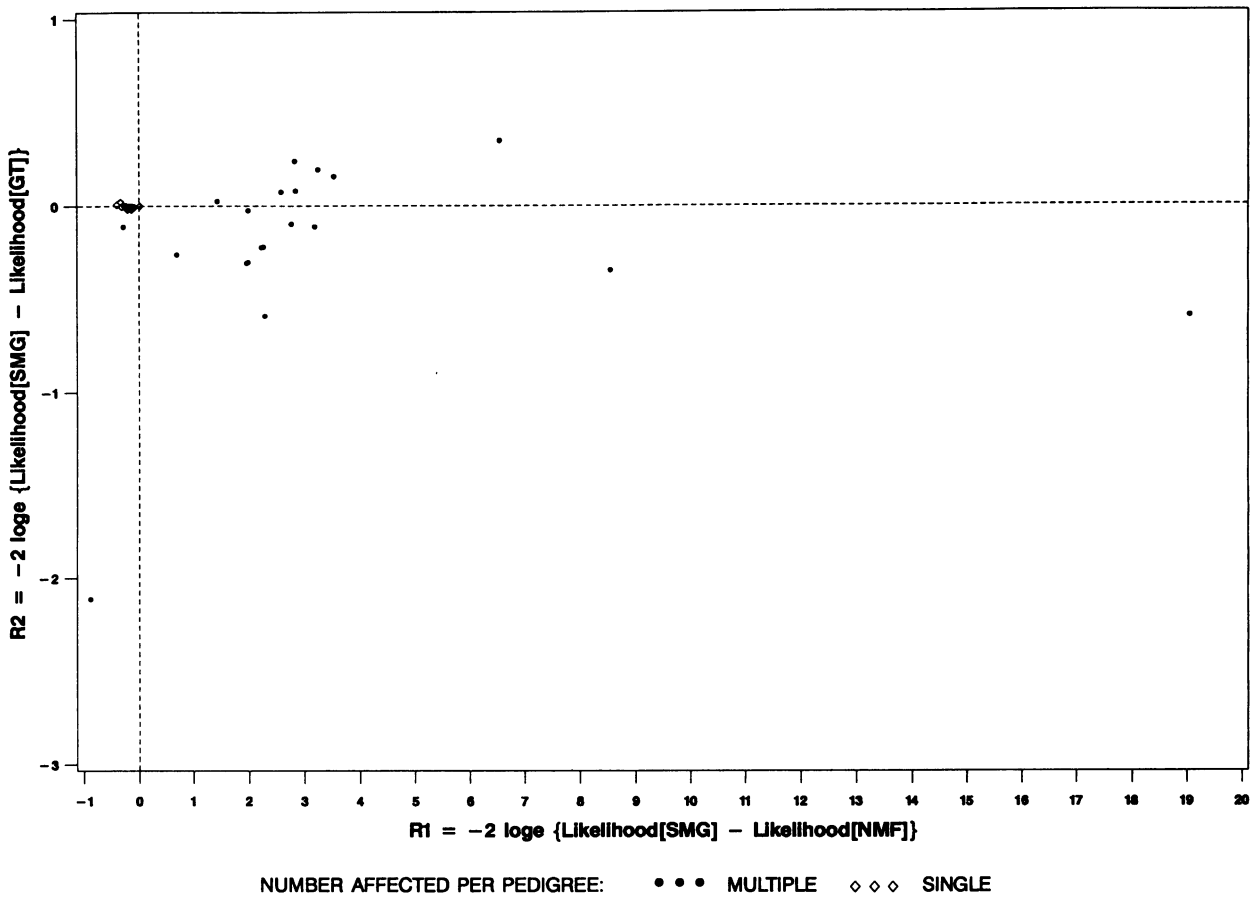

Figure 2 Bivariate likelihood-ratio comparison of SMG model with unrestricted genotype effects and sibling correlation (model 4 in table 6) with NMF and GT models with sibling correlation.

Discussion

The results of the present study indicate that an SMG and residual sibling correlation explain the probability of having ITEV in a sample of Caucasian pedigrees ascertained through probands with ITEV. This inference was made after the Mendelian inheritance hypothesis was not rejected and non-Mendelian hypotheses were rejected. In addition to the hypothesis testing strategy presented above, two other hypothesis testing strategies were undertaken. First, tests were made that reduced, in sequential order, the transmission parameterization, then the sibling correlation parameterization, and finally the penetrance function. Second, tests were made that reduced, in sequential order, the residual sibling correlation parameterization, then the transmission parameterization, and finally the penetrance function. Each of the three hypothesis testing strategies resulted in the identification of the same model (model 4 in table 6). The consistency of these results supports the inference that an SMG with residual sibling correlation explains the probability of having ITEV in this sample of pedigrees.

Descriptive analyses suggested that models containing parent-offspring correlation terms might be overparameterized with respect to the available data. The inclusion of parent-offspring correlation terms did not affect the inference of a single major gene or affect the point estimates of the remaining model parameters. This suggests that there was no bias or loss of information in our analysis as a result of not considering parent-offspring correlations in the models presented in tables 4–6. Furthermore, parental covariates contribute significantly to NTF and NMF models (although both are rejected in comparison with the GT model), while parental covariates do not contribute to any model containing a single gene (table 7). These results suggest that parent-offspring correlation may be completely ex-

plained by the vertical transmission of a single major gene.

The results of the present analyses describe the mode of inheritance of alleles, as well as frequencies and penetrances of genotypes, at the biometrically inferred ITEV gene. Of the models considered in the present study, the probability of having ITEV is best specified by the unrestricted effects of genotypes at a single autosomal Mendelian gene. This mode of inheritance is consistent with partial (incomplete) autosomal dominance of alleles. To assess the degree of dominance in these genotype effects, we computed $a = (\beta_{AA} - \beta_{BB})/2$ and $d = \beta_{AB} - (\beta_{AA} + \beta_{BB})/2$ (Falconer 1986, p. 101). The degree of dominance was expressed as $DD = d/a$. A value of $DD = -1$ indicates complete dominance of allele A over allele B. A value of $DD = 0$ indicates complete additivity of allelic effects. These values were computed to be $a = 9.822$, $d = -7.838$, and $DD = -0.798$. These values indicated that the disease allele A is partially (incompletely) dominant to allele B in this sample. The segregation of alleles at this gene may therefore be influenced by some other factor(s) that alters genotype penetrances.

The complex segregation analysis presented here considered only autosomal models of inheritance. By inspection, the majority of multiply affected pedigrees in this sample did not appear to support a sex-linked mode of inheritance (Thompson and Thompson 1980, pp. 69–76). The pattern of ITEV affection in the general population is more consistent with a sex-linked recessive model than with a sex-linked dominant model, since the incidence in males is much higher than the incidence in females. However, a number of pedigrees demonstrated patterns of transmission that were inconsistent with a recessive sex-linked model. These included father-son transmission ($N=3$), affected males not related through females ($N=3$), or mother-son transmission ($N=2$). Only 5 of the 20 multiply affected families in this sample are consistent with sex-linked recessive transmission. All five of these pedigrees also support the best-fitting SMG model (fig. 2). Therefore, it is very unlikely that a sex-linked model would be supported by complex segregation analyses in the present sample. As a result, this hypothesis was not tested directly in the model-fitting procedure presented here.

In order for there to be a distinction between all potential different genetic models in a likelihood-based complex segregation analysis, there must be expected observations in each unmeasured genotype class from which genetic parameters can be estimated. For example, to distinguish the likelihoods of recessive or dominant genetics models from an additive genetic model, individuals must be observed in the AA genotype class. Were this class of data missing, we might question whether the likelihood differences presented in table 5 resulted from differences in true maximum-likelihood solutions or whether these likelihood differences were due to nonconvergent solutions. We therefore examined whether matings involving the AA genotype class existed in the present sample.

This examination was accomplished by computing the probability of each possible mating type M_i (i = AA×AA, AA×AB, AA×BB, AB×AB, AB×BB, BB×BB) with an affected offspring, O. This probability was computed as $\Pr(M_i|O) = \Pr(O|M_i)\ \Pr(M_i)/\Sigma_i\ \Pr(O|M_i)\ \Pr(M_i)$ using the estimates of allele frequencies and genotypic penetrances in model 4 of table 6. Matings of the AA×AB type were most likely affected × unaffected, since the penetrance of the AA genotype was 1 and the penetrance of the AB genotype was .001. Eight affected × unaffected matings producing affected offspring were observed in the present sample (see fig. 1). We estimated that the AA×AB mating type occurred in the present sample with a probability of 6.4 matings/1,000. Given that 167 matings produced affected offspring in the present sample, we expect at least one of these to be an AA×AB mating (.0064 × 167). The relative frequency of the B allele (.991; model 4 in table 6) indicated that AB and BB genotypes were common in this sample. Consequently, matings involving each of the unmeasured genotype classes (AA, AB, and BB) occurred in the present sample.

Penetrances of genotypes at the biometrically inferred ITEV gene adjusted for sex-specific population incidences of ITEV were computed to be $e^{\beta_i}/(1+e^{\beta_i})$, where β_i (i = AA, AB, or BB) was the genotype-specific regression coefficient. These values were computed here to be 1.0, .039, and .0006, using the estimates of β_{AA}, β_{AB}, and β_{BB}, respectively, from model 4 (table 6). Wang et al. (1988) estimated penetrances to be .494, .020, and 0 in males and .358, .008, and 0 in females given the AA, AB, and BB genotypes, respectively. The penetrances estimated by Wang et al. are less than half those estimated in the present study for the AA genotype class. Averaged across gender, the penetrance estimated by Wang et al. is roughly half that estimated in the present study for the AB genotype class. Both the present study and that of Wang et al. estimate the penetrance in the BB genotype class to be at or near zero. Two study differences may explain this discrepancy: first, the estimate of the disease allele frequency in the present study was an order of magnitude lower ($P = .007 \pm .003$) than that of Wang et al. ($P = .03$); second, two very different genetic models were used to

generate these results. Both the present study and that of Wang et al. infer the segregation of a single (partially) dominant gene. However, the penetrance estimates differ substantially between these two studies.

While the goal of the present analyses was not to estimate recurrence risks of ITEV, the present data allow such an estimate to be made. The empirical recurrence risk for siblings of an affected proband was estimated by computing the proportion of affected siblings of probands in this sample. This computation assumed that recurrence risk was independent of birth order. For siblings of male probands, the recurrence risk was 2.0% (2 affected proband siblings of 101 proband siblings). For siblings of female probands, the recurrence risk was 0.7% (1 affected proband sibling of 141 total proband siblings). These values are lower than the proportion of affected siblings of probands reported in other Caucasian populations (Palmer et al. 1974; Czeizel et al. 1981; Wynne-Davies et al. 1982; Cartlidge 1984). Without respect to gender, those authors estimated recurrence risks of 5%–7% in proband siblings. All of the estimates are associated with wide confidence intervals, such that there is no statistically significant difference in these estimates. The previous studies are heterogeneous with respect to clinical diagnostic criteria, sampling design, and analytical methods. Therefore, those risk estimates may not be directly comparable to one another or to that of the present study. Furthermore, the present estimates considered only affected siblings of probands, while other studies considered affection in other relatives. Despite these differences, the recurrence risk to siblings of probands in all studies is substantially higher than any reported population rate of ITEV.

The results of the present study suggest that a substantial proportion of ITEV in the reference Caucasian Iowa population can be explained by the segregation of an SMG plus sibling correlation. Estimates of the prevalence of ITEV explained by the biometrically inferred ITEV gene were computed as $P_i\,[e^{\beta_i}/(1+e^{\beta_i})]$, where P_i is genotype frequency, and β_i (i = AA, AB, or BB) is the regression coefficient from the regressive logistic models. These values were computed to be 5×10^{-5} in the AA genotype class, 5.4×10^{-4} in the AB genotype class, and 5.4×10^{-4} in the BB genotype class. The sum of these values indicates that a rate of 1.13×10^{-3} ITEV cases in the general population are attributable to the single gene. In comparison with a reported population incidence of 1.2×10^{-3} (Palmer et al. 1974), about 94% of the ITEV in the general population is explained by the segregation of the SMG identified here.

By using the figures in the preceding paragraph, the proportion of cases attributable to each genotype can be determined using the equation $100\% \times P_i[e^{\beta_i}/(1+e^{\beta_i})]/\Sigma_i P_i[e^{\beta_i}/(1+e^{\beta_i})]$. These values were computed to be 4.4%, 47.8%, and 47.8% for the AA, AB, and BB genotype classes, respectively. Therefore, while the majority of ITEV in the population may be explained by the segregation of a single gene, roughly half of the cases of ITEV occur in individuals who do not carry the disease allele. The data available to the present study do not allow us to examine what factors other than the single gene may be responsible for ITEV.

The rate of ITEV affection in males has been reported in numerous studies to be about double that in females (Kite 1964; Chung et al. 1969; Palmer et al. 1974; Yamamoto 1979; Cartlidge 1984). In the present study, a sample of nearly 15,000 unrelated individuals was added to the analysis to adjust the point estimates in the logistic regressive model to more accurately reflect the sex-specific rates of ITEV affection reported by Palmer et al. (1974). The observed proportion of affected males:females in the sample of pedigrees was 1.8:1. When no single major factor was considered (e.g., the NMF model in table 4), the logistic regressive model did accurately reflect sex-specific population ITEV rates. When a single major gene was included in the regressive logistic model, the odds ratio associated with sex was estimated to be 1.9:1. A single-degree-of-freedom hypothesis test indicated that this odds ratio was not different from 1, in the presence of a single major gene (table 6). However, the sample that contributed to the information about sex differences in the segregation of a single major gene was relatively small. Eighteen of the 20 multiply affected families in the present sample gave evidence for the segregation of a single gene (fig. 2). In this subset of the total sample, there were only 24 affected males and 14 affected females (a male:female affection ratio of 1.7:1). The consistency of the observed male:female ratio of affection and the odds ratio estimates associated with sex suggest that the statistical power available in the present sample was not sufficient to detect this relatively small preponderance of male affection in the present sample after a single major gene and population rates of ITEV affection were considered in a logistic regressive model.

By using the results of the present study as well as knowledge from clinical and basic science research, it is possible to speculate about the identity of this biometrically inferred gene. Clinical evidence suggests that a disruption of the normal developmental sequence or a regional growth disturbance may explain the pathogenesis of ITEV. Clinical studies reveal that the leg and foot in ITEV are invariably small (Dietz 1986; Laaveg and

Ponseti 1980). The more severe the deformity, the more marked the reduction of foot and leg size. In addition, after it has been corrected, ITEV recurs during the rapid growth period of the foot.

These observations have led to a number of hypotheses about the pathogenesis of ITEV. ITEV may result from growth of the tissues of the anterolateral foot growing around the stunted posteromedial foot (Dietz et al. 1983). Support for this hypothesis is of two types. First, a disproportionate amount of type I muscle fibers in the posterior and medial muscle groups and in several peroneal muscles of clubfoot legs have been observed (Isaacs et al. 1977; Handelsman and Badalamente 1981). This suggests that a regional neural abnormality may be present, since muscle fiber type is neurally determined. This hypothesis is further supported by the knowledge that the region of the foot and leg that appears stunted is the area subtended by the tibial nerve. Second, comparisons of cellular characteristics in anterior and posterior tibial tendon sheaths in normal legs with those in ITEV specimens have shown that the posterior sheath in ITEV had fewer fibroblasts and smaller cell and cytoplasmic volume (Dietz et al. 1983). This suggests cellular hypoplasia.

Another hypothesis is derived from the observation that the "position" of the skeletal elements in ITEV is abnormal. Differentiation of tissues in ITEV proceeds normally, and no stage of normal foot development has the malposition seen in ITEV. This suggests that the signals that provide positional information for ITEV limbs are defective. Much is known about the determinants of cellular positional information. The anterior-posterior axis of the limb is in part determined by a gradient of retinoic acid (Tabin 1991). Evidence exists that retinoic acid (Wright et al. 1989; Tabin 1991) may act by inducing a sequence of homeobox genes, such as in the mouse muscle-segment-homeobox model (Holland 1991). The proximal/distal axis of the limb may be determined by a transforming-growth-factor-β gradient that stimulates integrins. This may provide positional information by increasing cell adhesion (Tabin 1991). A complex interaction of morphogens, growth factors, and homeobox genes is probably necessary for informing cells and tissues of their proper location in the developing limb. A defect in this system might result in the malpositioning of tissues in ITEV.

The finding that ITEV can be explained by the segregation of an SMG plus residual sibling correlation has important implications for researchers seeking the cause of idiopathic ITEV. The present results suggest that the search for the molecular genetic basis of this disorder is appropriate. The data presented here plus the availability of families likely to be segregating for this SMG provide a reasonable starting point in the search for a candidate ITEV gene using genetic linkage analyses and DNA association studies.

Genetic linkage studies have successfully localized a number of human Mendelian disorders of morphogenesis, including several for which a specific gene has been isolated. These include Waardenburg syndrome (PAX3; Morrell et al. 1992), Stickler syndrome (COL2A1; Francomano et al. 1987), and Grieg syndrome (GLI3; Vortkamp et al. 1991). Similarly, association studies using candidate genes support a role for transforming growth-factor α in nonsyndromic cleft lip and palate (Ardinger et al. 1989). Over 250 nonchromosomal syndromes include ITEV as a component (Computer Power Group 1991), and the genetic mapping of some of these (e.g., diastrophic dysplasia to chromosome 5q; Hastbacka et al. 1990) may identify target regions of the human genome or specific candidate genes. In addition, the appearance of ITEV as a common feature of many unbalanced chromosomal rearrangements (e.g., 4p trisomy and 7q monosomy; de Grouchy and Turleau 1986) suggests target genomic regions for linkage studies or inspection for candidate genes. This application of clinical, epidemiological, biostatistical, and molecular genetic approaches should eventually provide essential information for the identification of the gene inferred in the present study. The identification of this gene should ultimately improve the treatment and prevention of ITEV.

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