1237

Environmental and genetic factors in etiology of orofacial clefts in Argentina. *M.M.Tolarova[1], A.C.Goldberg[2], A.Capozzi[3], M.Pastor[4], R.Guillen[4], and R.P.M.Steegers-Theunissen[5].* University of California San Francisco[1],Marin General Hospital[2],St.Francis Mem.Hospital[3],California;Hospital A.Scaravelli,Tunuyan,Argentina[4],University Hospital Nijmegen St Radboud,The Netherlands[1].

Orofacial clefts (OFC) are common birth defects affecting about 1-2 in every 1,000 newborns worldwide. Genetic and environmental factors play a role in their etiology. Historical and recent studies suggest that a certain proportion of OFC could be prevented by periconceptional supplementation of FA and multivitamins. In order to analyze dietary information, key life style data and other epidemiological information, we carried out a case control study in Mendoza, Argentina. Altogether, 140 families of probands affected with OFC and 110 control families were analyzed.During personal interviews of mothers a detailed questionnaire consisting of 58 specific questions was filled out.All probands and controls and all their available relatives were examined by medical geneticist. Blood was obtained for determination of FA and vitamin B_{12} levels and for genotyping of 677C→T MTHFR gene. Of the 140 probands, 80 were males and 60 were females;11(6 males and 5 females) had cleft palate and 129 cleft lip with or without cleft palate. In 12 cases (8.6%),the OFC was part of a syndrome or a complex of multiple malformations.Both cases and controls came from the middle or low social class. In the group of cases, there was a higher number of fathers unemployed or working as seasonal workers.There was no significant difference in alcohol use or smoking found between mothers of cases and controls. For cases, a mean maternal age was 35.3, a mean paternal age 39.8,a mean birth weight 3,352 grams. Average size of sibship in proband families was 4.0, what was higher than in control families. Data on socioeconomical status, diet composition, other life-style information, blood levels of FA and vitamins were compared between cases and controls.In general, the diet of families of probands was much simpler than in controls. The results of our study suggest important role of exogenous factors in the etiology of OFC in Argentina.

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1238

Associations between lutelnizing hormone-β gene polymorphism and skeletal maturation and growth before and during puberty. *B. Towne[1], J.S. Parks[2], J. Blangero[3], L. Almasy[3], M.R. Brown[2], T.C. Murphy[2], E.W. Demerath[1], A.F. Roche[1], R.M. Siervogel[1].* [1]Wright State University, Dayton, OH; [2]Emory University, Atlanta, GA; [3]Southwest Foundation for Biomedical Research, San Antonio, TX.

Ralvio et al. (1997 JCE&M 81:3278-82) reported that Finnish boys heterozygous with a common variant β-subunit of the lutelnizing hormone (LH-β) allele (arg8, thr15) were shorter and had slower growth rates during puberty than boys homozygous for the wild-type LH-β allele (trp8, ile15). We examined associations between LH-β genotype determined by PCR and measures of skeletal maturation and growth in 241 children in the Fels Longitudinal Study. A measured genotype analysis was performed, allowing for sex-specific trait means, using the computer package SOLAR (Blangero and Almasy 1996). There were 111 boys and 96 girls homozygous for the wild-type LH-β allele, and 24 boys and 10 girls heterozygous with the variant allele.

Individual growth curves were fitted to serial childhood stature data using the triple logistic method implemented in the program AUXAL (Bock et al. 1994). Age at prepubertal minimum height velocity (APPHV) and age at pubertal peak height velocity (APHV) were derived, as were height and height velocity at those ages. These children also had serial skeletal maturity assessments made using the FELS method (Roche et al. 1988), from which skeletal age at APPHV and APHV were interpolated.

A pattern of significant, or near significant, associations were found between LH-β genotype and skeletal maturity and height both before and during puberty. LH-β heterozygotes were shorter than LH-β homozygotes at APPHV (p=0.057), and had younger skeletal ages (p=0.059). Similarly, LH-β heterozygotes were shorter than LH-β homozygotes at APHV (p=0.077), and had younger skeletal ages (p=0.036). No significant associations were found between LH-β genotype and APPHV, APHV, or height velocity at APPHV or APHV. Thus, in our sample of boys and girls, the effects of this LH-β polymorphism on both skeletal maturity and height are apparent at the onset of the pubertal growth spurt, and remain evident at the time of maximal pubertal growth.

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1239

Testing association between candidate-gene markers and phenotype in related individuals, by use of estimating equations. *David-Alexandre Trégouët, Pierre Ducimetière, and Laurence Tiret.* INSERM Unité 258, Paris, France.

Association studies are one of the major strategies for identifying genetic factors underlying complex traits. In samples of related individuals, conventional statistical procedures are not valid for testing association, and maximum likelihood (ML) methods have to be used, but they are computationally demanding and are not necessarily robust to violations of their assumptions. Estimating Equations (EE) offers an alternative to ML methods, for estimating association parameters in correlated data. We have studied through simulations the behavior of EE in a large range of practical situations, including samples of nuclear families of varying sizes and mixtures of related and unrelated individuals. For a quantitative phenotype, the power of the EE test was comparable to that of a conventional ML test and close to the power expected in a sample of unrelated individuals. For a binary phenotype, the power of the EE test decreased with the degree of clustering, as did the power of the ML test. This result might be partly explain by a modeling of the correlations between responses that is less efficient than that in the quantitative case. In small samples (<50 families), the variance of the EE association parameter tended to be underestimated, leading to an inflation of the type I error. The heterogeneity of cluster size induced a slight loss of efficiency of the EE estimator, by comparison with the balanced samples. The major advantages of the EE technique are its computational simplicity and its great flexibility, easily allowing investigation of gene-gene and gene-environment interactions. It constitutes a powerful tool for testing genotype-phenotype association in related individuals.

1240

LIMB GIRDLE MUSCULAR DYSTROPHY WITH CALPAIN DEFICIENCY IN GUIPUZCOA (BASQUE COUNTRY, SPAIN). *Urtizberea JA (1), Urtasun M (3), Richard I (2), Saenz A(3), Poza JJ(3), Cobo AM(3), Lopez de Munain A(3), Beckmann JS(2).* (1) AFM : 1, rue de l'Internationale 91000 EVRY - FRANCE (2) Genethon : 1, rue de l'Internationale - 91000 EVRY - FRANCE (3) Hospital N/S Aranzazu - 20080 - SAN SEBASTIAN - BASQUE COUNTRY - SPAIN

A survey of limb girdle muscular dystrophy has been carried out in 1995-1996 in the Basque province of Guipuzcoa (702,000 inhabitants). The diagnostic criteria were those agreed at the ENMC workshop held in Naarden in 1995. All patients were reexamined by two neurologists and underwent electrophysiological and immunohistochemical studies whenever necessary. Serum CK, bidimensionnal echocardiography and respiratory evaluation were also performed in order to investigate phenotypic variations. Genetic studies were carried out at Genethon and were designed to demonstrate a possible linkage to chromosome 15 (LGMD2A locus) or to test known mutations. A primary screening enabled to select 62 patients out of which 8 turned out to have an alternative diagnosis (2 with Becker muscular dystrophy, 3 with SMA, 1 with myotonic dystrophy, 1 with a-sarcoglycanopathy, 1 with an undetermined myopathy), and 4 were not available for the study. For the remaining 51 patients (29 men, 22 women, mean age 38.9 years ± 14.24), the clinical presentation was consistent with previous descriptions of LGMD in patients reported by M. Fardeau in the Réunion. A molecular screening for CANP3 revealed that 29 families corresponding to 38 patients were harbouring a mutation in the calpain gene while the status of the 13 other patients is pending. Interestingly, 18 families were homozygous for a mutation in exon 22 whereas 8 families were heterozygous for exon 22 and 1 family was homozygous for exon 21. The prevalence rate of LGMD 2A found in this Basque population (68/100,000) is one the highest so far reported worldwide. This cluster of patients, mostly of Basque origin, is presumably due to socioeconomical (inbreeding, no admixture with neighbouring spanish people) and geographical factors. The existence of one main mutation in exon 22 suggest a founder effect. Correlations between genotype and phenotype are still under way and demonstrates some similarities in the exon 22 mutation carriers and some discrepancies for the others.

1241

Presenilins and early-onset Alzheimer's Disease *C.M. van Duijn[1], M. Cruts[2], H. Backhovens[2], J. Theuns[2], G. Van Gassen[2], A. Hofman[1], C. Van Broeckhoven[2].* [1]Department of Epidemiology & Biostatistics, Erasmus University Rotterdam, The Netherlands, [2]Neurogenetics Laboratory, Flanders Interuniversity Institute for Biotechnology, Born-Bunge Foundation, University of Antwerp (UIA), Belgium.

Presenilin 1 (PS-1) and Presenilin 2 (PS-2) mutations have been implicated in autosomal dominant forms of early-onset Alzheimer's disease (AD). We have conducted a population-based association study in 102 patients with early-onset (before age 65 years) AD and 118 age- and sex matched controls. For PS-1, two polymorphisms were studied, one in the promoter region and one in the 5'UTR region (exon 1B). For PS-2, no polymorphisms in the promoter or 5'UTR region were found. Three polymorphisms in exon 3 and 4 and intron 11 of PS-2 were studied. The PS-1 promoter polymorphism was associated to AD (p=0.01; likelihood ratio test). The 2-allele of the promoter polymophism was found to be 2.5 times increased in AD patients (95% confidence interval: 1.2-5.4). An increased frequency was also found if carriers of known PS-1 mutations were excluded. Frequencies of the 5'UTR polymorphism in PS-1 (exon 1B) and PS-2 polymorphisms in exon 3 and 4 and intron 11 were similar in cases and controls. Earlier, we reported an association with two polymorphisms flanking the PS-1 promoter polymorphism: at intron 8 (two-fold increase of the 22 genotype; p=0.01) and at an upstream marker D14S1028 (3.5-fold increase of the 44 genotype; p=0.0005). Together with the present finding of an association with the promoter polymorhpmism and early-onset AD, the findings of our population-based studies suggest the presence of a (common) mutation in the promotor region of PS-1. At the population level, we and others have found no evidence for a major role of PS-2 in AD.

1242

Genetic association of two chromosome 14 genes (PS1 and ACT) with Alzheimer's disease. *X. Wang, S.T. DeKosky, C.E. Aston and M.I. Kamboh.* University of Pittsburgh, Pittsburgh, PA.

Alzheimer's disease (AD) is a multifactorial disease with the possible involvement of several genetic and environmental factors. Among the genetic factors, the gene coding for apolipoprotein E has been implicated with the risk of AD. In this study we have examined the relationship of two candidate genes on chromosome 14, including presenilin1 (PS1) and α 1-antichymotrypsin (ACT), with the risk of late-onset AD. The study subjects included 322 late-onset AD patients and 216 controls. The two polymorphisms examined were an intronic polymorphism in the PS1 gene and a signal peptide polymorphism in the ACT gene. Although the overall PS1 genotype distribution showed borderline difference between cases and control (p=0.08), the frequencies of PS1/2-1 (53.1% vs. 47.2% p=0.03) and PS1/2-2 (15.2% vs 22.7%; p=0.001) genotypes were significantly different between cases and controls. At the ACT locus the frequency of the *ACT*A* allele was significantly higher in cases than controls (0.556 vs. 0.473; p=0.006). The stratification of PS1 data by ACT genotypes showed that the risk associated with the *PS1*1* allele was confined to *ACT*A* carriers only (p=0.038). Similarly, the risk associated with the *ACT*A* allele was restricted to *PS1*1* carriers only (p=0.004). The two-site haplotype data showed that the A1 haplotype carrying the *ACT*A* and *PS1*1* alleles was more frequent in patients than controls (0.316 vs. 0.245; p=0.009). These data indicate a possible synergistic effect of these two loci on the risk of AD.