Letters to the Editor

Am. J. Hum. Genet. 66:2013–2014, 2000

High Frequency of Cystic Fibrosis Transmembrane Regulator Mutation L997F in Patients with Recurrent Idiopathic Pancreatitis and in Newborns with Hypertrypsinemia

To the Editor:

Cystic fibrosis (CF) (MIM 219700) is a genetic disease with multisystem involvement and in which defective chloride transport across membranes causes dehydrated secretion. The protein encoded by the CF gene (CFTR) is a transmembrane conductance regulator. The ability to detect CFTR mutations has led to the recognition of its association with a variety of conditions, including bronchiectasis, sinusitis with polyps, and male infertility (Estivill et al. 1996). A high frequency of mutations in the CFTR has more recently been shown in patients with chronic idiopathic pancreatitis (Sharer et al. 1998) and in newborns with hypertrypsinemia (Castellani et al. 1999). The exocrine pancreas is almost invariably affected in CF, even if not always with clinical manifestations (Lebenthal et al. 1993). In CF and in idiopathic pancreatitis, the earliest pathological finding is probably pancreatic ductular obstruction due to inspissated secretions (Oppenheimer et al. 1975; De Angelis et al. 1992). Hypertrypsinemia is thought to derive from pancreatic ductular obstruction and leakage of trypsinogen into the bloodstream (Crossley et al. 1979). Therefore, we postulated that there might be particular CFTR gene mutations involved in pancreatic ductular obstruction, as manifested in idiopathic pancreatitis or in neonatal hypertrypsinemia. Since routine CF mutation testing may miss rare gene alterations that can occur in these CF-related pathologies, a complete screening of the CFTR gene was performed in a group of 32 patients with idiopathic pancreatitis (14 of whom carried a CF mutation—the 5T variant—or borderline sweat chloride level, and 18 of whom were without common CF mutations or any other CF characteristic) and in 49 newborns with hypertrypsinemia and normal sweat chloride (32 of whom had a common CF mutation [some of these reported by Castellani et al. 1999], and 17 of whom did not have a common CF mutation). The 27 exons of the CFTR gene and their intronic flanking regions were analyzed by denaturing gradient-gel electrophoresis (DGGE) and by automatic sequencing, as described elsewhere (Bombieri et al. 1998).

Aside from some common CFTR mutations, none of which appeared to be more represented than expected, in comparison with the CFTR gene mutation distribution in CF patients in the same population, rare mutations were found in 9 of 32 patients with idiopathic pancreatitis and in 21 of 49 newborns with hypertrypsinemia. Among these rare mutations, L997F was identified in 4 (12.5%) of 32 patients with idiopathic pancreatitis (genotypes L997F/ΔF508, L997F/5T, and twice L997F/no mutation identified, respectively), and in 4 (8%) of 49 newborns with hypertrypsinemia (genotypes L997F/G542X, L997F/R553X, L997F/ΔF508, and L997F-F1052V phase unknown, respectively). The cumulative frequency of the L997F mutation in pancreatic dysfunction (8 [9.87%] of 81) is significantly higher than that found in normal control individuals (Bombieri et al. 1998, 2000, and unpublished data) from the same population (3 [0.97%] of 315; Fisher's exact test, $P = .0002$; odds ratio 11.397 (range 2.95–44.029). L977 is a highly conserved residue in transmembrane domain 9 among five species analyzed (Tucker et al. 1992). L997F was initially reported as a DNA variant (Fanen et al. 1992) and was described in a 5-year-old boy from northern India who presented a borderline sweat chloride value and features highly suggestive of CF (Cystic Fibrosis Genetic Analysis Consortium) and in patients with disseminated bronchiectasis (Bombieri et al. 2000, Girodon et al. 1997). Following the guidelines of a recent consensus statement on the diagnosis of CF (Rosenstein et al. 1998), we tested for L997F in 100 carriers of mutation ΔF 508 (mothers of typical patients with CF) from the same population as the individuals with pancreatitis and hypertrypsinemia; since none of them carried it, L997F is designated as a CF-causing mutation, according to criterion number 4 in the above-cited article (Rosenstein et al. 1998). This classification implies that the L997F heterozygotes compounded with common CF mutations found among the patients with idiopathic pancreatitis (ΔF508) and that the hypertrypsinemic newborns with negative sweat chloride (ΔF508, G542X, and R553X) should be diagnosed as affected by an atypical form of CF; a close follow-up is indicated for these patients. In conclusion, these data indicate that CFTR

L997F is associated with increased susceptibility to pancreatic ductular obstruction.

Acknowledgments

This work was supported by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica and by the Italian Ministry of Health, CF Project, law 548/93.

M. Gomez Lira,[1] M. G. Benetazzo,[1] M. G. Marzari,[1] C. Bombieri,[1] F. Belpinati,[1] C. Castellani,[3] G. C. Cavallini,[2] G. Mastella,[3] and P. F. Pignatti[1]

[1]*Section of Biology and Genetics, Department of Mother and Child, Biology and Genetics, and* [2]*Department of Surgical and Gastroenterological Sciences, University of Verona, and* [3]*Cystic Fibrosis Center, Ospedale Civile Maggiore, Verona*

Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Cystic Fibrosis Genetic Analysis Consortium, http://www.genet.sickkids.on.ca./cftr

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for CF [MIM 219700])

References

Bombieri C, Benetazzo MG, Saccomani A, Bepinati F, Gilè LS, Luisetti M, Pignatti PF (1998) Complete mutational screening of the CFTR gene in 120 patients with pulmonary disease. Hum Genet 103:718–722

Bombieri C, Giorgi S, Carles S, De Cid R, Belpinati F, Tandoi C, Pallares-Ruiz, et al (2000) A new approach for identifying non-pathogenic mutations: an analysis of the cystic fibrosis transmembrane regulator gene in normal individuals. Hum Genet 106:172–178

Castellani C, Benetazzo MG, Bonizzato A, Pignatti PF, Mastella G (1999) Cystic fibrosis mutations in heterozygous newborns with hypertrypsinemia and low sweat chloride. Am J Hum Genet 64:303–304

Crossley JR, Elliott RB, Smith PA (1979) Dried-blood spot screening for cystic fibrosis in the newborn. Lancet 1:472–474

De Angelis C, Valente G, Spaccapietra M, Angonese C, Del Favero G, Naccarato R, Andriulli A (1992) Histological study of alcoholic, nonalcoholic, and obstructive chronic pancreatitis. Pancreas 7:193–196

Estivill X (1996) Complexity in a monogenic disease. Nat Genet 12:348–350

Fanen P, Ghanem N, Vidaud M, Besmond C, Martin J, Costes B, Plassa F, et al. (1992) Molecular characterization of cystic fibrosis: 16 novel mutations identified by analysis of the whole cystic fibrosis conductance transmembrane regulator (CFTR) coding regions and splice junctions. Genomics 13:770–776

Girodon E, Cazeneuve C, Lebargy F, Chinet T, Costes B, Ghanem N, Martin J, et al (1997) CFTR gene mutations in adults with disseminated bronchiectasis. Eur J Hum Genet 5:149–155

Lebenthal E, Lerner A, Rolston DDK (1993) The pancreas in cystic fibrosis. In: Go VLW, DiMagno EP, Gardner JD, Lebenthal E, Reber HA, Scheele GA, eds. The pancreas: biology, pathobiology, and disease, 2d ed. Raven Press, New York, pp 1041–1081

Oppenheimer EH, Esterly JR (1975) Pathology of cystic fibrosis: review of the literature and comparison with 146 autopsied cases. Perspect Pediatr Pathol 2:241–278

Rosenstein BJ, Cutting GR (1998) The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 132:589–595

Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M, Braganza J (1998) Mutations of cystic fibrosis gene in patients with chronic pancreatitis. N Engl J Med 339:645–652

Tucker SJ, Tannahill D, Higgins CF (1992) Identification and developmental expression of the Xenopus laevis cystic fibrosis transmembrane conductance regulator gene. Hum Mol Genet 1:77–82

Address for correspondence and reprints: Dr. Macarena Gomez Lira, Section of Biology and Genetics, Department of Mother and Child, Biology and Genetics, University of Verona, Strada Le Grazie 8, I-37134 Verona, Italy. E-mail: macarena@borgoroma.univr.it

0002-9297/2000/6606-0035$02.00

Am. J. Hum. Genet. 66:2014–2015, 2000

On the Age of the Most Prevalent Gaucher Disease–Causing Mutation, N370S

To the Editor:

We have recently described a common origin for the most prevalent mutation (N370S) observed among Gaucher disease (GD) patients of Ashkenazi Jewish (AJ) and Spanish descent (Díaz et al. 1999). We also estimated the age of this mutation, using a formula described by Risch et al. (1995*b*). Unfortunately, as R. Colombo pointed out in a recent report (Colombo 2000), there was an error in the formula presented in the original publication that was never rectified. In a reply (Risch et al. 1995*a*) to criticisms raised by Zoossmann-Diskin (1995), Risch et al. made no mention of an error in the formula. The continued application of the formula by researchers who may not be well versed in the field may lead to repeated mistakes if the error remains uncorrected. We apologize for our failure to recognize the error, but we are pleased that it has been identified by Colombo, who had no difficulty in re-estimating the age of the mutation, using our data. This

reappraisal indicates that the N370S mutation may have occurred between the 11th and 13th centuries or even in the 10th century, considering 30 years per generation as suggested recently by Tremblay and Vezina (2000). This new estimated date for the mutation suggests that it occurred (or entered) the AJ population after the separation of the Ashkenazi and Sephardic Jewish traditions, which would be consistent with the apparent absence of this mutation among Sephardic patients. Using a totally different approach based on mutation detection in healthy Roman Jews, Oddoux et al. (1999) also got to the conclusion that the N370S is an old mutation. Further information on the origin of a mutation could be provided from the length of the chromosomal region noted with linkage disequilibrium (LD) and the strength of the LD. In this case, the data can be used to determine whether the N370S mutation had a Jewish or non-Jewish origin. The 3.2-cM region in LD in AJ (Díaz et al. 1999) seems to be shorter in Spanish chromosomes, as the flanking marker D1S2624 is in LD among AJ, whereas it is not in Spanish GD patients. Moreover, LD values are stronger in AJ than in Spanish chromosomes. These observations suggest that the mutation was introduced later in the AJ population, a statement that is independent of the actual age of the mutation. However, these results should be taken with caution because of the small number of Spanish chromosomes included in the study. Further work would be necessary to settle this issue which, to date, remains an open question.

Anna Díaz,[1] Magda Montfort,[1] Bru Cormand,[1] Baijin Zeng,[3] Gregory M. Pastores,[3] Amparo Chabás,[2] Lluïsa Vilageliu,[1] and Daniel Grinberg[1]

[1]*Departament de Genètica, Universitat de Barcelona, and* [2]*Institut de Bioquímica Clínica, Mejia Lequerica s/n, Barcelona; and* [3]*Department of Neurology and Pediatrics, NYU School of Medicine, New York*

References

Colombo R (2000) Age estimate of the N370S mutation causing Gaucher disease in Ashkenazi Jews and European populations: a reappraisal of haplotype data. Am J Hum Genet 66:692–697

Díaz A, Montfort M, Cormand B, Zeng BJ, Pastores GM, Chabas A, Vilageliu L et al (1999) Gaucher disease: the N370S mutation in Ashkenazi Jewish and Spanish patients has a common origin and arose several thousand years ago. Am J Hum Genet 64:1233–1238

Oddoux C, Guillen-Navarro E, Ditivoli C, Dicave E, Cilio MR, Clayton CM, Nelson H, et al (1999) Mendelian diseases among Roman Jews: implications for the origins of disease alleles. J Clin Endocrinol Metab 84:4405–4409

Risch N, DeLeon D, Fahan S, Ozelius L, Breakfield X, Kramer P, Almasy L, et al (1995*a*) ITD in Ashkenezi Jews—genetic drift or selection? In reply. Nat Genet 11:14–15

Risch N, DeLeon D, Ozelius L, Kramer P, Almasy L, Singer B, Fahn S, et al (1995*b*) Genetic analysis of idiopathic torsion dystonia in Ashkenazi Jews and their recent descent from a small founder population. Nat Genet 9:152–159

Tremblay M, Vezina H (2000) New estimates of intergenerational time intervals for the calculation of age and origin of mutations. Am J Hum Genet 66:651–658

Zoossmann-Diskin A (1995) ITD in Ashkenazi Jews—genetic drift or selection? Nat Genet 11:13–14

Address for correspondence and reprints: Dr. Daniel Grinberg, Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal, 645, 08071 Barcelona, Spain. E-mail: danielr@porthos.bio.ub.es

0002-9297/2000/6606-0036$2.00