J Med Genet 1998;35:82–87

LETTERS TO THE EDITOR

Cystic fibrosis and mucins

More than 600 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) have been identified in people with cystic fibrosis (CF Genetic Analysis Consortium, http://www.genet.sickkids.on.ca). There is wide variation in disease severity in CF that cannot be explained by CFTR genotype alone, though some degree of correlation between genotype and phenotype is possible. The ΔF508 mutation is generally associated with a relatively severe phenotype[1]; however, there is still a wide spectrum of disease severity seen among people carrying the same combination of mutations, whether as homozygotes or compound heterozygotes.[2] This has led to investigation of the possible causes of this variation. One possibility is that disease severity is genetically determined by other modifier loci that may have a direct effect on CF phenotype. Evidence for modifier loci has recently been observed in a strain of CF "knockout" mice.[3] As in most other CF mouse models the majority of null mutants die within 10 days after birth or at weaning from severe intestinal disease. A genome scan in a subgroup of these mice that survived weaning and showed prolonged survival indicated that a gene(s) in the proximal region of mouse chromosome 7 might be involved in this milder phenotype. Candidate genes in the syntenic region of human chromosome 19q13 include subunits of protein kinase C, Na^+/K^+ATPase, and a sodium channel. Some of these may interact with the cAMP activated chloride ion channel encoded by the CFTR protein and hence might be relevant to modifier effects in CF pathology.

Another potential candidate gene family that might be relevant to phenotypic variation in CF are the mucin genes. Mucins play a role in the pathology of CF: they contribute to the obstruction of small airways following lung infection; intestinal obstruction that is often associated with CF involves mucins, particularly the meconium ileus that affects 15% of CF newborns; and mucins contribute to blocked ducts that result in pancreatic destruction during the second and third trimester of gestation. The molecular identities of proteins in mucus were poorly characterised until the isolation of a number of human mucin genes enabled the identification of those that are expressed in different epithelia. Nine human mucin genes have been identified, MUC1, 2, 3, 4, 5AC, 5B, 6, 7, and 8.[4] There is little sequence homology among different types of mucin gene sequences, though one common feature of almost all mucin genes that has come to be considered a motif for mucin is the tandem repeat. Mucin tandem repeats (TR) are regions of primary sequence (nucleic acid and amino acid) that contain several tandemly arrayed identical (or highly similar) repeats of shorter sequence elements. Mucin TRs generally contain a high percentage of serine and threonine residues that are predominant sites of O linked glycosylation. These tandem repeats are highly polymorphic with the repeat number showing mendelian inheritance for all mucin genes examined to date.[5]

One hypothesis to account for mucin abnormalities in CF is that the fluid secretion defects in CF are solely responsible for mucin dehydration. However, we wish to propose an alternative hypothesis: the biophysical and biochemical properties of a mucin molecule will be affected by its TR length. Hence, the TR length may be relevant to the mucin abnormalities seen in CF. We wished to test the hypothesis that mucins are candidates for CF modifier loci.

CF is characterised by pancreatic insufficiency in all but 10-15% of patients. This is caused by ductal obstruction in utero causing gradual autolysis of the pancreas as the acini become functional between 20 and 24 weeks of gestation.[6] Though some data show that pancreatic sufficiency is often associated with mutations other than ΔF508, many patients are homozygous for ΔF508 and pancreatic sufficient. Further, some homozygotes or compound heterozygotes for other defined mutations show substantial variation in pancreatic function. We recently identified the MUC6 mucin as a major component of pancreatic mucin expressed during the mid-trimester of human gestation,[7] the time at which pancreatic destruction begins because of deposition of periodic-acid Schiff positive material in the small pancreatic ducts. Moreover, MUC6 mucin is a significant constituent of the material that obstructs the CF fetal pancreatic ducts.[8] The biochemical properties of the MUC6 mucin, including TR length, might contribute to the formation of ductal deposits.

Therefore, we examined MUC6 tandem repeat length in CF patients carrying a number of different defined mutations and characterised with respect to their pancreatic disease status (pancreatic insufficient or sufficient). The aim of this study was to establish whether there was an association between MUC6 tandem repeat number and severity of pancreatic disease.

The MUC6 gene is in a cluster of four closely linked mucin genes located at 11p15.4-11p15.5.[9] The MUC6 cDNA has not been fully characterised but appears to be at least 15-16 kb. Similar to other mucins, it contains a serine and threonine rich TR sequence with an individual repeat unit of 507 bp and 169 amino acids. The TR sequence is polymorphic and several restriction endonucleases can be used to assess TR allele length. Because of the overall size of the TR, Southern blot analysis of *TaqI* digested genomic DNA was used to investigate MUC6 TR allele length. Blots were probed with a 74 bp fragment of MUC6 homologous to part of the tandem repeat (GGTCCACACACACAGCCCCACCAGTGACGCCGACCACCAGTGGGACGAGCCAAGCCGCGAGCTCATTCAGCACA) (bases 308-381 EMBL accession number L07517). DNA samples from 50 CF patients with defined CFTR mutations were analysed, a total of 100 chromosomes. Of these, six patients were pancreas sufficient (PS) based on the results of pancreatic function tests, and the remainder were pancreas insufficient (PI). The six PS CF patients carried the following genotypes: two were ΔF508 homozygotes, one was homozygous for the G85E mutation,[10] and the remainder were compound heterozygotes for ΔF508 and G551D,[11] 2789+5GA,[12] or W1098R,[13] respectively.

The distribution of MUC6 allele sizes was between 7 kb and 13 kb with a peak at 10 kb, these values being similar to those observed by others in a non-CF population (D Swallow, personal communication). The MUC6 TR allele sizes among the six PS CF patients lay within the same size distribution and followed a similar pattern of distribution with a peak at around 10 kb. Three of the PS subjects were homozygous for the MUC6 TR allele size, of 9.8 kb (G85E/G85E), 10.2 kb (ΔF508/G551D), and 11 kb (ΔF508/2789+5A) respectively. The other three had TR allele sizes of 8.3/9.8 (ΔF508/ΔF508), 7/11.2 (ΔF508/W1098R), and 9.8/11.2 (ΔF508/ΔF508). Results of the study showed that there was no correlation between the number of tandem repeats within the MUC6 mucin and pancreatic status in 100 CF chromosomes.

The results we obtained do not support the hypothesis that MUC6 is a modifier locus for CF. Nonetheless, it remains possible that an increase in mucin glycoprotein size, through allelic variation in the TR copy number, will affect the biophysical and biochemical properties of the mucin (the physical dimensions of MUC6 and the total number of potential O glycosylation sites being dependent on the number of tandem repeat units). Further studies are required to investigate the effect of TR repeat number on the viscoelastic and biochemical properties of mucin, particularly under conditions of different ionic strengths and levels of hydration.

Most epithelia that are involved in the pathology of CF secrete different combinations of mucin glycoproteins, with some tissue specificity.[4 7] For example, the MUC4 mucin gene is expressed in most epithelial cells in the trachea, bronchus, and bronchioles while the MUC5B mucin gene is expressed primarily in submucosal gland epithelial cells and their progenitors. A more extensive examination of TR variation in these and other mucin genes with respect to different aspects of CF disease pathology should be undertaken.

The authors thank Drs S Abbs, F Beards, and F Flinter and colleagues at the South East Thames Genetics Centre, London, UK for their help and Drs D Swallow and M A Hollingsworth for helpful discussions.

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1 Kerem E, Corey M, Kerem BS, *et al.* The relationship between genotype and phenotype in cystic fibrosis - analysis of the most common mutation ΔF508. *N Engl J Med* 1990;**323**:1517-22.
2 Dean M, Santis G. Heterogeneity in the severity of cystic fibrosis and the role of CFTR gene mutations. *Hum Genet* 1994;**93**:364-8.
3 Rozmahel R, Wilschanski M, Matin A, *et al.* Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. *Nat Genet* 1996;**12**:280-6
4 Reid CJ, Gould S, Harris A. Developmental expression of mucin genes in the human respiratory tract. *Am J Resp Cell Mol Biol* (in press).
5 Swallow DM, Gendler S, Griffiths B, Corney G, Taylor-Papadimitriou S, Bramwell ME. The human tumour-associated epithelial mucins are coded by an expressed hypervariable locus PUM. *Nature* 1987;**328**:82-4.
6 Harris A, Chalkley G, Goodman S, Coleman L. Expression of the cystic fibrosis gene in human development. *Development* 1991;**113**:305-10.
7 Reid CJ, Harris A. Developmental expression of mucin genes in the human digestive system. (Submitted.)

8 Reid CJ, Hyde K, Ho SB, Harris A. Cystic fibrosis of the pancreas: involvement of MUC6 mucin in obstruction of pancreatic ducts. *Mol Med* 1997;3:403–11.
9 Toribara NW, Robertson AM, Ho S, *et al.* Human gastric mucin. *J Biol Chem* 1993;**268**: 5879-85.
10 Zielenski J, Bozon D, Kerem B, Markiewicz D, Rommens JM, Tsui LC. Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 1991;**10**:229-35.
11 Cutting GR, Kasch LM, Rosenstein BJ, *et al.* A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator protein. *Nature* 1990; **346**:366-9.
12 Highsmith WE, Strong T, Burch N, *et al.* Identification of a splicing error in exon 14B giving rise to a frameshift mutation in a consanguineous family with mild cystic fibrosis. *Paediatr Pulmonol* 1990;**suppl** 5:11A.
13 Zielenski J, Markiewicz D, Chen HS, *et al.* Identification of six mutations (R31L, 441delA, 681delC, 1461ins4, W1098R, E1104X) in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Hum Mutat* 1995;5: 43-7.

Chinese geneticists approach ethics

Your recent editorial[1] expressed a concern that has been escalating in the international scientific community with respect to eugenic legislation in China and elsewhere.[2 3] China is a developing nation, with the world's largest population and oldest continuous civilisation. In 1994, China launched its Human Genome Project (HGP).[4] While Chinese geneticists are expressing an enthusiastic view of new genetic technology, including HGP and mammalian cloning, what are their views of ethical, legal, and social issues of genetics research and practice in China, particularly the Chinese eugenic law? Some western geneticists think that Chinese geneticists are keeping silent about these issues,[5] as their opinions on these have hardly been heard either in or outside China. This may be because Chinese geneticists have had very unhappy experiences, in which so-called politics have interfered with genetics research and education in the past.[6] Another explanation may be that psychologically Chinese prefer to express their views on sensitive issues indirectly or privately. An international survey on ethics and genetics conducted in 37 countries, including China,[7] however, has shown that 63% of Chinese geneticists are willing to express their views on ethical issues in China, although some of these views are quite different from those of western geneticists.[6 8-10]

The concept of non-directiveness, and the particular reasons for being non-directive, have only very slowly gained credibility in western genetics over the last 15 or 20 years (M Bobrow, 1997, personal communication). In 1985, when geneticists in 19 countries were asked whether they would be non-directive in counselling, 99% of them agreed.[11] However, when presented with actual cases in this survey, the majority of geneticists in Europe would counsel directively. In the United States, Canada, the United Kingdom, Australia, Denmark, Finland, Germany, Israel, the Netherlands, Norway, Portugal, and South Africa, substantial majorities said they would be "as unbiased as possible" in counselling for conditions such as Klinefelter syndrome (XXY), cystic fibrosis (CF), neurofibromatosis (NF), and familial hypercholesterolaemia (FH), which do not ordinarily involve mental retardation. In the US, Canada, UK, Australia, Finland, Germany, the Netherlands, and Norway, most would also be unbiased for trisomy 21. In the US, UK, and Norway most would also be unbiased in counselling for severe spina bifida (D C Wertz, 1995, presented at the annual conference of the European Society of Human Genetics). While most Chinese geneticists would prefer to practise directive counselling, most of them thought that 25 of 26 conditions listed in table 1 were severe enough to warrant termination of pregnancy.[10] It is hard at this stage to say whether geneticists practising in other cultures like China's will eventually move in the same direction of non-directive counselling, or whether there really are fundamental societal differences as opposed to just differences of timing (M Bobrow, 1997, personal communication).

As the survey shows, the permissiveness of Chinese geneticists is most striking in their willingness to tell others, such as insurers, employers, relatives, and spouses, about genetic testing results without necessarily having the donor's consent. This question of what to do with information about genetic susceptibility is one of the most difficult in western society, and such an approach would clearly be unacceptable in most European based societies, including the US (W Bodmer, 1997, personal communication). The main concerns of western geneticists relate to state direction of genetic decisions rather than individual choices,[12] while the survey shows that most Chinese geneticists hold strong social views on genetic decision making. In China, public education in genetics has lagged owing to lack of funding and expertise as well as a large number of illiterate and semi-literate people, while genetics training for medical professionals in China has also been insufficient. The number of genetics service providers in China is currently declining because it is less profitable than many other medical specialties. Moreover, the ethical, legal, social, and psychological implications of the new genetics have not yet been investigated because of lack of interest and funding. This is the present situation of genetics in China.

In 1985, Fletcher *et al*[13] proposed that geneticists around the world would benefit from collective reflection on their preferred approaches to the most frequent of the difficult moral choices in practical genetics. Based on the proposal, guidelines on ethical issues in genetics and the provision of genetics services have recently been recommended to the World Health Organisation.[14] The survey shows, however, that there has been less consensus on several major ethical issues between western geneticists and their counterparts in the developing countries, including China. Wide and intensive discussions are therefore necessary if these guidelines are to be adopted worldwide.

Table 1 Chinese geneticists' views on how to counsel about termination for 26 conditions

Condition	% Would			
	Urge termination	*Counsel pessimistically*	*Be unbiased*	*Counsel optimistically*
Anencephaly	93	5	1	1
Trisomy 13	91	7	1	1
Trisomy 21	90	7	2	1
Severe spina bifida	89	9	1	1
Hurler syndrome	85	12	2	2
Cystic fibrosis	82	13	4	1
Achondroplastic dwarfism	77	15	5	3
Mother's life in danger	77	12	8	3
45,X	74	19	5	2
Huntington's disease	73	21	4	2
XXY	72	20	4	4
PKU	68	18	8	6
Sickle cell anaemia	67	24	6	3
Rape	67	18	13	2
HIV infection in fetus	62	17	11	10
Toxoplasmosis in fetus	61	25	9	5
Neurofibromatosis	60	24	9	7
Rubella in fetus	57	26	12	5
Familial hypercholesterolaemia	56	30	8	6
Cleft lip and palate: girl/boy	52/48	28/26	12/15	8/11
Predisposition to mental illness	51	33	12	4
Severe obesity	31	25	27	17
Predisposition to alcoholism	29	43	19	9
Predisposition to Alzheimer's disease	27	40	22	11
Undesired sex	6	2	30	62

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1 Bobrow M. Redrafted Chinese law remains eugenic. *J Med Genet* 1995;**32**:409.
2 Editorial. China's misconception of eugenics. *Nature* 1994;**367**:1-2.
3 Editorial. Brave new now. *Nat Genet* 1997;**15**:1-2.
4 Li YQ. China launches genome project. *Nature* 1994;**365**:200.
5 O'Brien C. China urged to delay "eugenics" law. *Nature* 1996;**383**:204.
6 Mao X. Ethics and genetics in China: an inside story. *Nat Genet* 1997;**17**:20.
7 Wertz DC, Fletcher JC. Geneticists approach ethics: an international survey. *Clin Genet* 1993;**43**:104-10.
8 Mao X. Chinese ethics. *Nature* 1996;**384**:404.
9 Mao X. Chinese eugenic legislation. *Lancet* 1997;**349**:139.
10 Mao X, Wertz DC. China's genetic services providers' attitudes towards several ethical issues: a cross-cultural survey. *Clin Genet* 1997; **52**:100–9.
11 Wertz DC, Fletcher JC, Mulvihill JJ. Medical geneticists confront ethical dilemmas: cross-cultural comparisons among 18 nations. *Am J Hum Genet* 1990;**46**:1200-13.
12 Harper PS. Genetic counselling and society. In: *Practical genetic counselling*. 4th ed. Oxford: Butterworth-Heinemann, 1995:317-29.
13 Fletcher JC, Berg K, Tranoy KE. Ethical aspects of medical genetics. A proposal for guidelines in genetic counsel,prenatal diagnosis and screening. *Clin Genet* 1985;**27**:199-205.
14 Wertz DC, Fletcher JC, Berg K, Boulyjenkov V. *Guidelines on ethical issues in medical genetics and the provision of genetics services*. Hereditary Diseases Programme, Division of Noncommunicable Diseases, WHO, Geneva, 1995.