A p53 binding sites

Consensus	RRRCW	WGYYY	(N) ⁰⁻¹³	RRRCW	WGYYY
Human FAC sequence	¹⁸⁴⁸ AGACA	TGTTC	C	AGACC	AGTTC ¹⁸²⁸
Mouse FAC sequence	²⁰¹⁰ AGACA	TGTTC	C	AGACC	AGTTC ¹⁹⁹⁰

B Leucine zipper motifs

Consensus	LXXXXXXXXXXXXXXXXXXXXXXXXL-----LXXXXXXXXXXXXXXXXXXXXXXXXL
Human FAC sequence	³⁸ VAQFQEFRLRKMVEALKEMDSNT ⁵⁹ ----- ²³⁴ LPMSAVVCLWLRHLPLEKAMLHL ²⁵⁷
Mouse FAC sequence	³⁸ LSGFQEFRLRQMYEILKEMDSAIL ⁶¹ ----- ²³⁵ VPTSSVSVLSLWFRHLPLEKATLHL ²⁵⁸

repeats. The second of the zipper motifs is preceded both by a helix-turn-helix region (with the crucial glycine at position 208) and a distinctly basic domain (position 160 to 176). The picture that emerges (figure) is that of a prototype nuclear protein designed to interact both with DNA and proteins.⁹ The strong conservation between the murine¹⁰ and human motifs (table) argues for their functional significance: the conservation amounts to 100% for the p53 binding site motif (*v* 78% in the remainder of the sequence), and to 90% *v* 79% for the amino acid residues within the leucine zipper domains.

The apparent contradiction between nuclear¹¹ and cytoplasmic location^{12,13} of the FAC protein can be reconciled if one assumes that the inactive bulk of the protein is located in the cytoplasm, and that transfer into the nucleus and subsequent activation occurs only in response to DNA damage.

WOLFGANG LIEBETRAU
MANFRED BÜHNER
HOLGER HOEHN
Institut für Humangenetik,
Biozentrum, University of Würzburg,
Am Hubland, 97074 Würzburg,
Germany.

The COX8 gene is not the disease gene of the CMH4 locus in familial hypertrophic cardiomyopathy

Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant disorder characterised by ventricular hypertrophy which mainly affects the interventricular septum and causes severe myocardial and myofibrillar disarray.^{1,2} FHC is genetically heterogeneous. Four loci have been identified: CMH1 on chromosome 14, CMH2 on chromosome 1, CMH3 on chromosome 15, and CMH4 on chromosome 11, and a fifth locus exists.³ On these loci, three genes have been identified, respectively encoding the cardiac sarcomeric proteins β myosin heavy chain, troponin T, and α tropomyosin.^{4,5} For these three genes, there is also allelic heterogeneity.³ The gene implicated in the CMH4 locus⁶ is not yet known. We attempted to ascertain whether the gene COX8, which is localised at 11q12-q13 and encodes the subunit VIII of the cytochrome c oxidase complex (COX),⁷ is the CMH4 disease gene. COX is the terminal enzyme of the mitochondrial respiratory chain and participates in the production of ATP through oxidative phosphorylation.⁸ Therefore, an alteration of a gene encoding one of the COX subunits might modify ATP production in the cell, leading to a compensatory myocardial hypertrophy. Furthermore, several deficiencies in COX activity have been described in hypertrophic cardiomyopathies.^{9,10} All these characteristics make the COX8 gene a good candidate for the CMH4 locus, and analyses were performed on family 714 in which this locus was described.⁶

Since the genomic structure of the COX8 gene is not known, Southern blot analysis was carried out, as described in Schwartz *et al.*,¹¹ in a search for deletions or insertions or both, using COX8 cDNA as specific probe.¹² The DNA of 60 people, 11 of whom were affected, was analysed after digestion with 12 restriction enzymes (*Bam*HI, *Bcl*II, *Bgl*II, *Eco*RI, *Eco*RV, *Hind*III, *Hinf*I, *Msp*I, *Pst*I, *Pvu*II, *Rsa*I, and *Taq*I). The results showed no difference between the DNA of affected and healthy people as regards the length of the DNA fragments shown (data not shown), indicating that there were no major modifications in the genomic structure of the COX8 gene.

The next step was to look for alterations in the mRNA. Since the COX8 gene is ubiquitously transcribed,⁷ northern blot analyses were performed, using 10 μ g of total RNA purified from lymphoblastoid cell lines of four affected and four healthy people, and COX8 cDNA was used as specific probe.¹² These analyses showed no differences between the length of COX8 mRNA in healthy and affected members of the family. Individual COX8 mRNAs were further quantified, using 18S RNA as internal probe. The ratios of COX8 mRNA/18S RNA were as follows: for the affected subjects, 0.62, 0.69, 0.63, and 0.54 respectively, and for the healthy subjects, 0.55, 0.61, 0.66, and 0.70. These results showed no significant differences in mRNA levels. Consequently, the absence of major alterations in COX8 transcripts enabled us to exclude the possible presence of mutations in intronic splicing sites, which would have led to transcript deletion or insertion or both.

Lastly, COX8 transcripts were sequenced. Reverse transcription and amplification of COX8 mRNA (RT-PCR) were carried out with 1 μ g of total RNA purified from lymphoblastoid cell lines of four affected and four healthy people. The strategies of am-

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COX8 cDNA amplification and sequencing strategies. COX8 cDNA is depicted as described in Rizzuto *et al.*⁷ (A) Reverse transcription and amplification using poly-T primer and F 1-22 specific primer. (B) Amplification of cDNA 5' end using the 5'-RACE method: reverse transcription of COX8 cDNA with R 231-250 specific primer, followed by addition of a poly-C tail at the 5' end of this cDNA, on which what is termed a 5'-RACE primer is anchored, to amplify the DNA fragment with the R230-250 specific primer. Arrows indicate the different sequencing steps of DNA fragments A and B. The hitherto unidentified sequence of 42 nucleotides at the 5' end of the cDNA is also presented.

plification and sequencing are shown in the figure. A first strand cDNA synthesis kit (Pharmacia) with poly-T primer and specific COX8 primer (F 1–22) was used for cDNA amplification between nucleotides 22 and 472 (figure A), according to the nucleotide numbers reported by Rizzuto *et al.*⁷ The 5' end of COX8 cDNA, before nucleotide 22, was amplified with the 5'-RACE kit (Life Technology/Gibco-BRL) and specific COX8 primer (R 231–250, figure B). Overlapping PCR fragments were sequenced using the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides on an Applied Biosystem 373A DNA sequencer (Perkin Elmer/ABI). We found a hitherto unidentified sequence of 42 nucleotides at the 5' end of the cDNA, and the rest of the COX8 cDNA sequence was identical to that previously described by Rizzuto *et al.*⁷ (figure). From nucleotides –42 to +472, there were no mutations in either the affected or healthy people in the COX8 cDNA.

The promoter region of the COX8 gene, the part of the gene that has not yet been identified, was not analysed. However, all the mutations so far identified in FHC have been described, either in the coding regions of the gene (mutations in MYH7 encoding the β myosin heavy chain³), or in an intronic splicing site (mutation in the splice donor sequence of intron 15 of the cardiac troponin T gene⁵). By analogy with all the data available for FHC, we therefore conclude that the COX8 gene is very unlikely to be the disease gene of the CMH4 locus responsible for FHC.

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GISÈLE BONNE
LUCIE CARRIER
KETTY SCHWARTZ
Unité de Recherche 153 de l'INSERM,
Pavillon Rambuteau,
Groupe Hospitalier Pitié-Salpêtrière,
47 Boulevard de l'Hôpital,
75651 Paris CEDEX 13, France.
MICHEL KOMAJDA
Service de Cardiologie,
Groupe Hospitalier Pitié-Salpêtrière,
Paris, France.

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BOOK REVIEWS

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Assessing Genetic Risks: Implications for Health and Social Policy. Editors L B Andrews, J E Fullerton, N A Holtzman, A G Motulsky. Committee on Assessing Genetic Risks, Division of Health Sciences Policy, Institute of Medicine. (Pp 338; £36.95) Washington, DC: National Academy Press. 1994. ISBN 0-309-04798-6.

This fascinating report provides a comprehensive account of the "promises and problems in genetic testing". The deliberations of the 20 committee members were informed by a series of papers and discussions at workshops, meetings, and a public forum.

The first 28 pages comprises the Executive Summary and provides the reader with a useful synopsis of the 70 plus recommendations. Many of their recommendations mirror those found in the Nuffield report on ethical issues in genetic screening (London: Nuffield Foundation, 1993). One example is the creation of a National Advisory Committee Working Group on Genetic testing to oversee professional practice and determine when new genetic tests are ready for wide scale use in medical practices. The Nuffield report proposes the setting up of a central coordinating body to review genetic screening programmes and monitor their implementation and outcome.

The report covers the following issues: Genetic testing and assessment; Laboratory issues in human genetics; Issues in genetic counselling; Public education in genetics; Personnel issues in human genetics; Financing of genetic testing and screening; Social, legal, and ethical implications of genetic testing; and Research and policy agenda.

The numerous references for each of these topics are situated at the end of each chapter. The report continuously emphasises the need to respect the autonomy of people in the way they use genetic information.

I was delighted to see two pages devoted to "Recognizing Social and Cultural Differences". The report identified the need for a variety of information and education on genetics, with balanced descriptions, in a culturally acceptable manner and at an appropriate time. I found the term "teachable moment" a very useful concept (that is, when the person is most able to comprehend the full significance of the information). Recommendations about developing innovative information materials such as interactive computer systems were noted on several occasions. As in the Nuffield report, reference is made to the training needs of primary care practitioners.

It is of interest that the Chairman of the Committee (A G Motulsky) felt the need to add a separate note to the Preface. In it he pointed out that while the majority of the committee favoured voluntary participation in neonatal screening, a minority felt that mandatory screening for phenylketonuria (PKU) and hypothyroidism would be a simpler solution.

On a more personal note, he stated that information about sickle cell trait that is incidentally detected in neonatal screening is difficult to withhold and should be given to the mother with appropriate genetic counselling. This seems to be in contrast to the more confusing recommendation of the report: "When carrier status may be incidentally determined in newborn screening (eg, in sickle cell screening), parents should be informed in advance about the benefits and limitations of genetic information, and that this information is not relevant to the health of their child. If they ask for the results of the incidentally determined carrier status for their own reproductive planning, it should be communicated to them in the context of genetic counselling, and they should be informed that misattributed paternity could be revealed."

This has been a most enjoyable book to read and I would strongly recommend it to anybody interested in the broader issues raised by genetic screening and testing.

ELIZABETH N ANIONWU

Genetics in Neurology. Bailliere's Clinical Neurology. Editor A E Harding. (Pp 452; £27.50) UK: Harcourt Brace. 1994.

How much of this rapidly expanding field can you cover in 452 pages (including references and index)? To their credit, the editor and authors of this text have included all the inherited neurological diseases which are likely to be of major interest to clinical neurologists and clinical geneticists. In addition, judging by the number of times this reviewer's copy was borrowed, molecular geneticists working with these diseases will also find the chapters enlightening.

The chapters are all well written, comprehensible, and, in addition to providing excellent reviews of their subjects, provide an insight into the issues of genetic and phenotypic heterogeneity as they relate to the specific disease groups. The chapters on hereditary ataxias (Banfi and Zoghbi) and