International Journal of Cardiology 126 (2008) 439–442

International Journal of Cardiology

www.elsevier.com/locate/ijcard

Letter to the Editor

The search of 'novel' mtDNA mutations in hypertrophic cardiomyopathy: MITOMAPping as a risk factor

Hans-Jürgen Bandelt [a,*], Yong-Gang Yao [b], Antonio Salas [c,d]

[a] *Department of Mathematics, University of Hamburg, Bundesstr. 55, 20146 Hamburg, Germany*
[b] *Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, 650223 Kunming, Yunnan, China*
[c] *Unidad de Genética, Instituto de Medicina Legal, Facultad de Medicina, Universidad de Santiago de Compostela, 15782, Galicia, Spain*
[d] *Centro Nacional de Genotipado (CeGen), Hospital Clínico Universitario, 15706, Galicia, Spain*

Received 13 February 2007; accepted 16 February 2007
Available online 7 May 2007

Abstract

MITOMAP is by far the most frequently cited Web resource that is referred to in substantiating novelty of an mtDNA mutation. This database, as is now known, has quite an incomplete coverage of the mtDNA mutations from the literature. This circumstance has seduced many scholars of medical genetics in the past to claim novelty of rather 'worn-out' mtDNA mutations. What is, however, really novel in the field is that researchers take advantage of this situation and deliberately suppress information from other sources, as it appears to have occurred in two recently published cases of hypertrophic cardiomyopathy.

Keywords: Hypertrophic cardiomyopathy; Noonan syndrome; Mitochondrial DNA mutation; Haplogroup; MITOMAP

1. Introduction

How could one know whether an array of non-silent mtDNA mutations is implicated in a certain syndrome? Quite often, some anecdotal finding in a single case is regarded as the first evidence for pathogenicity of mtDNA mutations — turned into a publication, even without having carried out any functional analysis. Instead, the 'novelty' of a number of mtDNA mutations observed in the patient would be emphasized. Such a kind of approach, however, which is being exercised on an ever growing number of diseases, is beset with a conceptual shortcoming: there is no compelling evidence to date that, say, half a dozen of non-silent mtDNA mutations could realistically arise in the germline just within a few generations [1]. Rather, it would typically take several thousand years before such an array of mutations is attained in the matriline. Thus, there is a good chance that related mtDNA lineages bearing only a subset of those targeted mutations thrive in the patient's ethnic group or regional population. Then a focussed large-scale study could attempt to determine whether some particular mutation of that array is mildly deleterious or whether there is some synergetic effect of several mutations, possibly enhanced by interaction with nuclear DNA.

'Novelty' of mutations found in patients with certain diseases is often tagged to titles and abstracts of articles, which has probably enhanced their publication. Traditionally, MITOMAP (http://www.mitomap.org/) appears as the sole witness for the novelty of an mtDNA mutation. But straightforward Internet searches in the 'warehouse' of mitochondrial variation may quickly reveal that just the usual suspects of pathogenic mutations or the known polymorphisms have actually been revisited [2].

2. Hypertrophic cardiomyopathy (HCM)

In a recent study, entitled "*Novel mitochondrial DNA mutations in a rare variety of hypertrophic cardiomyopathy*", Prasad et al. [3] have claimed novelty of six mutations observed in an Indian HCM patient. A quick screening of the

* Corresponding author.
E-mail address: bandelt@math.uni-hamburg.de (H.-J. Bandelt).

0167-5273/$ - see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ijcard.2007.02.049

mtDNA literature allows us to re-evaluate their claim of novelty. Entering queries for these mutations in mtDB (http://www.genpat.uu.se/mtDB/) reveals that four of those mutations were published in population studies before the submission (July 2005) of the paper. A fifth mutation (C1556T) was not novel either, as one learns after entering the query '1556 mtDNA patient' into Google (Table 1).

Therefore only the mutation G3407A seemed to have been unknown at the stage of submission. But, the subsequent paper [4] exhibited a new mtDNA lineage (sample #OR89) of haplogroup M5a (with T12477C being diagnostic together with G709A, C3921T, and G14323A) possessing G3407A and T11365C as well. Therefore, it is very likely that the mtDNA of the patient analysed in [3] belongs to a branch of M5a that is defined by G3407A and T11365C. This means that the necessary publication order had been reversed, by first describing a single case and then systematically investigating mtDNA mutations in the general population.

3. Noonan syndrome

Dhandapany et al. [5] have studied the case of a Noonan patient with hypertrophic obstructive cardiomyopathy and sequenced the entire mtDNA, but did not disclose the corresponding complete mtDNA sequence which they claimed to have obtained. Instead, as many as eight 'novel' mutations were listed along with one mutation deemed to be associated with prostate cancer (according to MITOMAP, but see [2] for a critical assessment of some mutations claimed to be implicated in prostate cancer). In fact, all except two mutations (A10316G and C14436T) were known before 2005 and can conveniently be retrieved from the mtDB database (Table 1). The existence of the mtDB Web site could not really have escaped the attention of the corresponding author because his research group actually used this resource in another paper [6] that was submitted in December 2005 (accepted February 2006), well before the submission date (July 2006) of the subsequent paper [5].

Entering 'A10316G mtDNA' into Google yields the reference to [7], where this mutation was recorded in the sample #B177 belonging to haplogroup M4'30. The latter paper was cited in the paper by Thangaraj et al. [4], which was submitted in December 2005 and published in June 2006, thus prior to the submission of the paper [5]. Putting the meagre information offered in [5] into phylogenetic perspective, it is obvious that A2755G signifies the entire Indian mtDNA haplogroup R8, whereas three further mutations discussed there (A5510G, C5911T, and C13782T) define a branch of R8 in India [8]. Thus, there remains very little substance for constructing a disease association story.

4. Discussion

What was then novel in the studies [3] and [5]? Certainly not the polymorphisms of two particular branches of the familiar

Table 1
'Novelty' of mutations reported in [3] and [5]

Ref.	Position	Change	Gene	Occurrences in the literature [a]	MITOMAP tree [b]	Haplogroup [c]
[3]	869	C→T	12S rRNA	[9]; [10]; [11]; [12]	Yes (E)	E1 (*2*); L2c; ?
[3]	1391	T→C	12S rRNA	[13] (*2*); [14] (*3*); [15]; [8]; [16]; [17]	Yes (R1)	B4b1b (*3*); Q1 (*4*); R1 (*2*)
[3]	1556	C→T	12S rRNA	[18] (cf. [19])	No	?
[3]	3407	G→A	ND1	[4]	No	M5a
[3]	11365	T→C	ND4	[20]; [21]; [4]; [22]	Yes (D5;N)	A2b; D5a2; M5a; N22
[3]	12477	T→C	ND5	[23]; [24]; [9]; [20]; [14] (*3*); [25]; [26]; [7] (*6*); [4]; [27]; [28]; [29]; [30]	Yes (L1; M5; M*; M11)	J*; J2a1; K1a2; L1c1a (*2*); L1c2a (*2*); M5a (*9*); M11a; M33; Q1; X2
[5]	2755	A→G	16S rRNA	[9]; [20] (*2*); [14] (*4*); [8] (*3*); [10]; [7]; [38]	Yes (L1; M; R8)	L0a; L1c1a (*7*); M*; R8 (*3*); ?
[5]	5510	A→G	ND2	[8] (*2*)	Yes (R8)	R8 (*2*)
[5]	5911	C→T	COI	[31]; [9]; [14]; [32]; [8] (*2*); [10] (*4*); [39]	Yes (L0; R8)	H3b; L0a (*5*); L0a1; L0a1a; R8 (*2*); U
[5]	10283	A→G	ND3	[9]; [14]; [33] (*3*); [34]	No	H*; Q2 (*3*); U5b (*2*)
[5]	10316	A→G	ND3	[7]	Yes (M2)	M4'30
[5]	11172	A→G	ND4	[35]; [9]; [36]; [20] (*2*); [14] (*13*); [27]	Yes (L0)	K2c; L0a2 (*18*)
[5]	12127	G→A	ND4	[9] (*2*); [37] (*3*); [10]	Yes (L0)	J1c (*2*); L0a; M29 (*3*)
[5]	13782	C→T	ND5	[8] (*2*); [31]	Yes (R8)	H1a; R8 (*2*)
[5]	14436	C→T	ND6	–	No	–

[a] References to the articles where the referred mutations were also reported. Most of these instances were thus published before [3,5] and can be retrieved from the mtDB database. Multiple occurrences of a particular mutation in one publication are indicated by the italicized number in round brackets.

[b] MITOMAP tree column refers to the presence or absence of the corresponding variants in the worldwide phylogeny displayed at http://www.mitomap.org/mitomap-phylogeny.pdf; in round brackets we indicate the haplogroup status as it is designated in that tree.

[c] Haplogroups in which the corresponding mutation thrives; the italicized number in round brackets counts the sequences with that mutation in the corresponding haplogroup (when >1). A question mark indicates that haplogroup status could not be determined.

Indian haplogroups, M5a and R8, respectively. It is also unlikely that the remaining mutations are inflicted in HCM. One cannot firmly exclude the possibility, of course, that some mutation only sporadically observed otherwise might be involved in the disease. But in any case such an implication would need more evidence from solid and systematic analyses. Only one single mutation, C14436T, might really be considered to be novel, but this would not automatically entail its pathogenicity since many coding region sequences from population studies carry their own private mutations not seen anywhere else so far. Since mtDNA variability is high in populations, the discovery of new polymorphisms is to be expected when sequencing any mtDNA whatsoever, especially in populations characterized by high mtDNA diversity, which is not yet fully documented, as is the case for India.

It is quite a common malpractice in the field to analyse the entire mitochondrial genome without documenting the full variation (or storing it e.g. in GenBank) but instead reporting only a handful of mutations [1]. Worse, false claims of novelty of mutations, as blessed by MITOMAP, may misguide future research by setting the agenda for rather futile projects, such as the search for specific mtDNA mutations involved in the Noonan syndrome. In this way, unguided MITOMAPping can be a risk factor in exploring the genetic causes of HCM.

References

[1] Salas A, Yao Y-G, Macaulay V, Vega A, Carracedo Á, Bandelt H-J. A critical reassessment of the role of mitochondria in tumorigenesis. PLoS Med 2005;2:e296.

[2] Bandelt H-J, Salas A, Bravi CM. What is a 'novel' mtDNA mutation — and does 'novelty' really matter? J Hum Genet 2006;51:1073–82.

[3] Prasad GN, Vanniarajan A, Emmanuel C, Cherian KM, Singh L, Thangaraj K. Novel mitochondrial DNA mutations in a rare variety of hypertrophic cardiomyopathy. Int J Cardiol 2006;109:432–3.

[4] Thangaraj K, Chaubey G, Singh VK, et al. In situ origin of deep rooting lineages of mitochondrial macrohaplogroup M in India. BMC Genomics 2006;7:151.

[5] Dhandapany PS, Sadayappan S, Vanniarajan A, et al. Novel mithochonrial DNA mutauions implicated in Noonan syndrome. Int J Cardiol 2007;120:284–5.

[6] Vanniarajan A, Nayak D, Reddy AG, Singh L, Thangaraj K. Clinical and genetic uniqueness in an individual with MELAS. Am J Med Genet Part B 2006;141B:440–4.

[7] Sun C, Kong Q-P, Palanichamy Mg, et al. The dazzling array of basal branches in the mtDNA macrohaplogroup M from India as inferred from complete genomes. Mol Biol Evol 2006;23:683–90.

[8] Palanichamy Mg, Sun C, Agrawal S, et al. Phylogeny of mitochondrial DNA macrohaplogroup N in India, based on complete sequencing: implications for the peopling of South Asia. Am J Hum Genet 2004;75:966–78.

[9] Herrnstadt C, Elson JL, Fahy E, et al. Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences from the major African, Asian, and European haplogroups. Am J Hum Genet 2002;70:1152–71.

[10] Parson TJ (unpublished). GenBank accession numbers: DQ304897, DQ304898, DQ304899, DQ304899, DQ304900, DQ304903, and DQ304987.

[11] Martin AM, Hammond E, Nolan D, et al. Accumulation of mitochondrial DNA mutations in human immunodeficiency virus-infected patients treated with nucleoside-analogue reverse-transcriptase inhibitors. Am J Hum Genet 2003;72:549–60.

[12] Trejaut JA, Loo J-H, Lin M (unpublished). GenBank accession number: EF093547.

[13] Tanaka M, Cabrera VM, González AM, et al. Mitochondrial genome variation in eastern Asia and the peopling of Japan. Genome Res 2004;14:1832–50.

[14] Kivisild T, Shen P, Wall DP, et al. The role of selection in the evolution of human mitochondrial genomes. Genetics 2006;172:373–87.

[15] Ingman M, Gyllensten U. Mitochondrial genome variation and evolutionary history of Australian and New Guinean aborigines. Genome Res 2003;13:1600–6.

[16] Yamasoba T, Goto Y, Oka Y, Nishino I, Tsukuda K, Nonaka I. Atypical muscle pathology and a survey of *cis*-mutations in deaf patients harboring a 1555 A-to-G point mutation in the mitochondrial ribosomal RNA gene. Neuromuscul Disord 2002;12:506–12.

[17] Pereira L, Gonçalves J, Franco-Duarte R, et al. No evidence for an mtDNA role in sperm motility: data from complete sequencing of asthenozoospermic males. Mol Biol Evol 2007;24:868–74.

[18] Tanimoto H, Nishio H, Matsuo M, Nibu K-I. A novel mitochondrial mutation, 1556C>T, in a Japanese patient with streptomycin-induced tinnitus. Acta Otolaryngol 2004;124:258–61.

[19] Fischel-Ghodsian N. Genetic factors in aminoglycoside toxicity. Pharmacogenomics 2005;6:27–36.

[20] Ingman M, Kaessmann H, Pääbo S, Gyllensten U. Mitochondrial genome variation and the origin of modern humans. Nature 2000;408:708–13.

[21] Kong Q-P, Yao Y-G, Sun C, Bandelt H-J, Zhu C-L, Zhang Y-P. Phylogeny of East Asian mitochondrial DNA lineages inferred from complete sequences. Am J Hum Genet 2003;73:671–6.

[22] Macaulay V, Hill C, Achilli A, et al. Single, rapid coastal settlement of Asia revealed by analysis of complete mitochondrial genomes. Science 2005;308:1034–6.

[23] Coble MD, Just RS, O'Callaghan JE, et al. Single nucleotide polymorphisms over the entire mtDNA genome that increase the power of forensic testing in Caucasians. Int J Legal Med 2004;118:137–46.

[24] Finnilä S, Lehtonen MS, Majamaa K. Phylogenetic network for European mtDNA. Am J Hum Genet 2001;68:1475–84.

[25] Malyarchuk BA, Grzybowski T, Derenko MV, Czarny J, Miścicka-Śliwka D. Mitochondrial DNA diversity in the Polish Roma. Ann Hum Genet 2006;70:195–206.

[26] Rajkumar R, Banerjee J, Gunturi HB, Trivedi R, Kashyap VK. Phylogeny and antiquity of M macrohaplogroup inferred from complete mt DNA sequence of Indian specific lineages. BMC Evol Biol 2005;5:26.

[27] Torroni A, Achilli A, Macaulay V, Richards M, Bandelt H-J. Harvesting the fruit of the human mtDNA tree. Trends Genet 2006;22:339–45.

[28] Kösel S, Grasbon-Frodl EM, Mautsch U, et al. Novel mutations of mitochondrial complex I in pathologically proven Parkinson disease. Neurogenetics 1998;1:197–204.

[29] Zhao L, Young WY, Li R, et al. Clinical evaluation and sequence analysis of the complete mitochondrial genome of three Chinese patients with hearing impairment associated with the 12S rRNA T1095C mutation. Biochem Biophys Res Commun 2004;325:1503–8.

[30] Povalko N, Zakharova E, Rudenskaia G, et al. A new sequence variant in mitochondrial DNA associated with high penetrance of Russian Leber hereditary optic neuropathy. Mitochondrion 2005;5:194–9.

[31] Achilli A, Rengo C, Magri C, et al. The molecular dissection of mtDNA haplogroup H confirms that the Franco–Cantabrian glacial refuge was a major source for the European gene pool. Am J Hum Genet 2004;75:910–8.

[32] Maca-Meyer N, González AM, Larruga JM, Flores C, Cabrera VM. Major genomic mitochondrial lineages delineate early human expansions. BMC Genet 2001;2:13.

[33] Friedlaender J, Schurr T, Gentz F, et al. Expanding Southwest Pacific mitochondrial haplogroups P and Q. Mol Biol Evol 2005;22:1506–17.

[34] Annunen-Rasila J, Finnilä S, Mykkanen K, et al. Mitochondrial DNA sequence variation and mutation rate in patients with CADASIL. Neurogenetics 2006;7:185–94.
[35] Behar DM, Metspalu E, Kivisild T, et al. The matrilineal ancestry of Ashkenazi jewry: portrait of a recent founder event. Am J Hum Genet 2006;78:487–97.
[36] Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N. Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proc Natl Acad Sci U S A 1995;92:532–6.
[37] Merriwether DA, Hodgson JA, Friedlaender FR, et al. Ancient mitochondrial M haplogroups identified in the Southwest Pacific. Proc Natl Acad Sci U S A 2005;102:13034–9.
[38] Wani A, Sharma N, Shouche YS, Bapat SA. Nuclear-mitochondrial genomic profiling reveals a pattern of evolution in epithelial ovarian tumor stem cells. Oncogene 2006;25:6336–44.
[39] Petros JA, Baumann AK, Ruiz-Pesini E, et al. mtDNA mutations increase tumorigenicity in prostate cancer. Proc Natl Acad Sci U S A 2005;102:719–24.