Letters to the Editor

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Errors, Phantom and Otherwise, in Human mtDNA Sequences

To the Editor:

The good news is that a very large number of human mtDNA sequences from diverse populations and ethnic groups are becoming available for analysis. The bad news is that many of these sequences contain errors (Dennis 2003; Forster 2003). In at least one instance, that of the Icelandic population, it appears that mtDNA sequence errors were a contributing factor (although not the only one) to an erroneous conclusion about the genetic diversity of these people (Arnason 2003). Forster (2003) cites other examples where mtDNA sequence errors have compromised analyses of population genetics and human evolution. In a reanalysis of mtDNA sequences in the Ladin population of the Alps, the original conclusions on population diversity were not overturned after the use of more accurate sequences (Vernesi et al. 2002). At this point, we do not know the extent of the damage, so to speak, caused by mtDNA sequence errors. Nevertheless, it is clear that correcting such errors must be undertaken as quickly as possible.

As a result of our reduced median network analyses (Herrnstadt et al. 2002), we released a database of 560 human mtDNA coding region sequences. A small number of errors in these sequences were detected by Dr. Hans-Jürgen Bandelt, and we were able to correct these, as noted in an erratum that was published soon after our original report (Herrnstadt et al. 2002). Subsequently, a systematic approach to the detection of phantom sequence errors was published in this *Journal* (Bandelt et al. 2002). As defined by these investigators, phantom errors are those that arise during the sequencing process itself. Dr. Bandelt contacted us again and suggested that there were phantom mutations in our mtDNA database. Specifically, the likely errors involved G→C transversions at nt 7927 and nt 7985. Such a result was surprising to us, because we believed that our sequencing approach and quality control measures had avoided such errors. Therefore, we used Dr. Bandelt's information as a starting point for a comprehensive reanalysis of our database.

After reanalysis, which included inspection of the electropherograms for all G→C and C→G transversions, we found that 41 of these mtDNA sequences contained at least one such phantom error. In fact, there were more such phantom errors than those suggested by Dr. Bandelt. In addition to the phantom transversions at positions 7927 and 7985, we detected instances of other such errors that included ones at nucleotide positions 500, 14160, 14460, 14974, and 16239. However, these errors did not occur randomly throughout the database. Instead, we could "isolate" the errors to a short time period that was relatively early during our large-scale mtDNA sequencing program. With the benefit of hindsight, it appears that the frequency of these errors was caused by two technical factors (see also Bandelt et al. 2002). The first was that one particular capillary array of the ABI 3700 DNA Analyzer produced suboptimal base separations, whereas the second was that the sequencing chemistry at that time utilized an early version of reagents that was optimized subsequently.

In addition to these 41 sequences, we also found that an additional 26 mtDNA sequences contained errors that arose during data entry or editing. As a result of this reanalysis, we have corrected the database of 560 sequences, which is available through the MitoKor Web site (the URL address is given below).

Have these errors invalidated our network analyses? Not to a substantial degree. Many of the sequence errors generated private polymorphisms, which were not included in our analyses. Furthermore, a substantial proportion of the branches in these networks were established by multiple substitutions (see figs. 1–4 in Herrnstadt et al. 2002), and, so far, we have no evidence from additional network analysis that the original results need major revision. Can we now guarantee that our mtDNA database is error free? No. Although such is our goal, it is not practical, and it is probably not technically feasible.

It is now clear that many mtDNA databases or sequence sets contain errors (Forster 2003). The solution to this problem is further effort, both at the front end (the sequencing process itself) and at the back end (increased quality control) of mtDNA database construction.

Acknowledgments

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Electronic-Database Information

The URL for data presented herein is as follows:

MitoKor, http://www.mitokor.com/science/560mtdnasrevision.php (for the revised 560 mtDNA coding-region sequences; "zip" and "sit" files also available)

References

Arnason E (2003) Genetic heterogeneity of Icelanders. Ann Hum Genet 67:5–16

Bandelt H-J, Quintana-Murci L, Salas A, Macaulay V (2002) The fingerprint of phantom mutations in mitochondrial DNA data. Am J Hum Genet 71:1150–1160

Dennis C (2003) Error reports threaten to unravel databases of mitochondrial DNA. Nature 421:773–774

Forster P (2003) To err is human. Ann Hum Genet 67:2–4

Herrnstadt C, Elson JL, Fahy E, Preston G, Turnbull DM, Anderson C, Ghosh SS, Olefsky JM, Beal MF, Davis RE, Howell N (2002) Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian, and European haplogroups. Am J Hum Genet 70:1152–1170 (erratum 71:448–449)

Vernesi C. Fuselli S, Castri L, Bertorelle G, Barbujani G (2002) Mitochondrial diversity in linguistic isolates of the Alps: a reappraisal. Hum Biol 74:725–730

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South Asia, the Andamanese, and the Genetic Evidence for an "Early" Human Dispersal out of Africa

To the Editor:

The out-of-Africa model of anatomically modern human evolution posits an African origin 100,000–200,000 years ago, followed by subsequent dispersal(s) to Eurasia and other continents within the last 100,000 years (Stringer and Andrews 1988). Although alternative models have been proposed, the out-of-Africa scenario receives the most support both from archeological and genetic evidence (Lahr and Foley 1994). However, the route(s) followed by the African migrants remain poorly understood. One proposed route was through northern Africa toward the Levant, which finds support in the archeological and fossil records (Lahr and Foley 1994). This exit of modern humans out of Africa would have taken place during the Upper Paleolithic era (~45,000 years ago), which considerably postdates the earliest evidence of modern human presence in the Sahul. Indeed, luminescence dating, paleovegetation changes, and skeletal remains suggest that Australia was inhabited by modern humans by 60,000 years ago (Roberts and Jones 1994; Johnson et al. 1999; Miller et al. 1999; Thorne et al. 1999), implying a substantially earlier migration from Africa to Australia. To take this evidence into account, as well as morphological and archeological features of many Australian fossils, a second migration of modern humans, known as the "southern route" hypothesis, was suggested to have occurred during Middle Paleolithic times (60,000–100,000 years ago) from eastern Africa to Sahul via South Asia (Cavalli-Sforza et al. 1994; Lahr and Foley 1994).

In the January 2003 issue of the *Journal,* Endicott et al. (2003) investigated the genetic affinities of 11 Andaman islanders, a group of people in the Indian Ocean with phenotypic similarities to some African populations (i.e., "Negrito" features) and reputed to be possible descendants of early migrants out of Africa to Sahul, following the southern route. The authors claim that the results of their investigation "support the growing evidence of an early movement of humans through southern Asia." In our opinion, Endicott and colleagues' results do not support any relationship between the present Andamanese population and the hypothesized early southern migration. The authors identified three different mtDNA haplotypes in 11 Andaman islanders, two belonging to haplogroup M2 and one belonging to M4. These haplogroups had previously been reported only in the Indian subcontinent (Kivisild et al. 1999*b*; Bamshad et al. 2001). The Andaman M4 haplotype has been found previously in mainland India (Kivisild et al. 1999*b*), whereas the two Andaman M2 haplotypes are (so far) unique to the Andamanese. Given that (1) the latter two types occupy a basal position in the M2 network, which has an estimated coalescence time of 63,000 ± 6,000 years (Kivisild et al. 1999*b*), and (2) they are not found in mainland India, Endicott et al. (2003) conclude they represent an "early" settlement of the Andaman Islands. These two points need discussion.

Regarding point 1, the age of a haplogroup cannot be automatically equated to the age of subsets of this haplogroup. The founding type of haplogroup M2, characterized by 16223T and 16319A relative to the Cambridge reference sequence (CRS) (Anderson et al. 1981) (fig. 1),

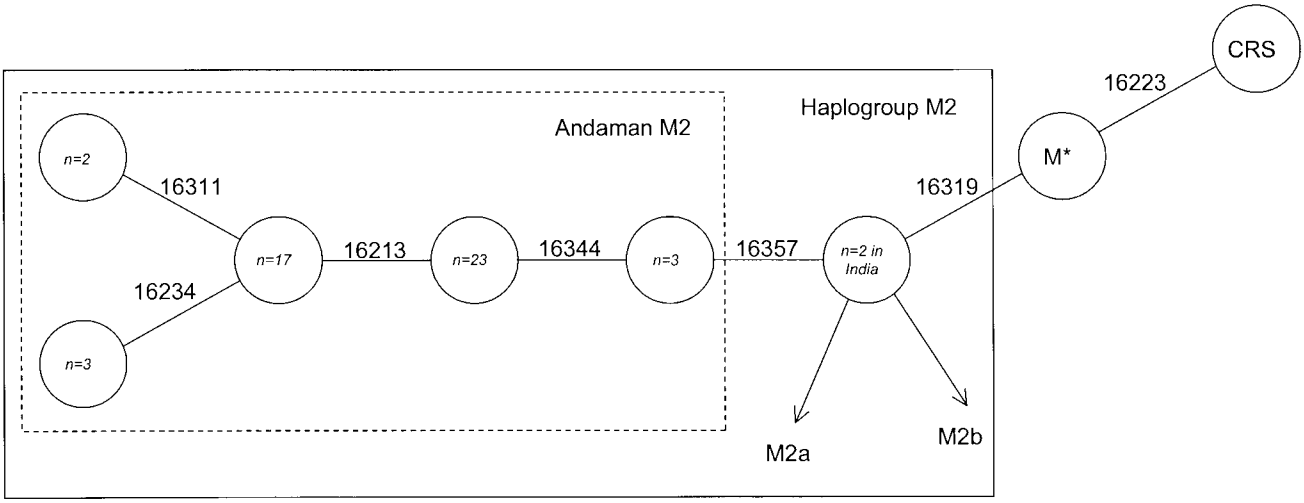

Figure 1 Median joining network (after Endicott et al. 2003) of mtDNA haplogroup M2. Substitution positions relative to the CRS are shown. The number of assigned individuals from sampling to date is indicated in the relevant nodes.

is dated to 63,000 years but is still present in mainland India. This does not mean that any cluster branching off of this node is 63,000 years old, but rather that it is, *at most,* 63,000 years old. In principle, the Andaman M2 cluster could be dated to any time between 63,000 years and today.

Regarding point 2, we do not question the fact that the Andaman M2 haplotypes have not been found in mainland India. However, related sequences are found in mainland India, one of which is cited by Endicott et al. (2003) as a candidate ancestral haplotype of the Andaman M2 sequences, since it differs by only one substitution at 16274 from one Andaman M2 haplotype. From figure 2 of Endicott et al. (2003), we identify in mainland India another candidate ancestral haplotype for the Andaman M2 types, namely, the founder type of the entire M2 haplogroup (characterized by 16223T, 16319A). This haplotype differs from one Andaman haplotype by a single substitution (at 16357) and has already been reported in mainland India (Kivisild et al. 1999*b*). Therefore, although identical haplotypes are not found elsewhere, the Andaman M2 haplotypes have closely related counterparts in present-day mainland India.

The extent of isolation experienced by Andaman islanders (and, thus, an approximate upper time limit for the colonization of the archipelago) can be estimated by dating the time needed to accumulate the observed variation of the Andaman-specific mtDNA lineages. In this respect, we analyzed 42 unambiguously determined mtDNA sequences from Onge, Jarawa, and Great Andamanese individuals (Thangaraj et al. 2003) and six sequences from Aka-Bea individuals (Endicott et al. 2003) belonging to haplogroup M2 (on the basis of the motif 16223T/16319A). Altogether, the 48 Andaman M2 sequences defined five haplotypes unified by 16357C (fig. 1). First, we investigated the pattern of demographic history for the data set of 48 Andaman M2 sequences, by calculating Fu's F_s (Fu 1997) and Tajima's D (Tajima 1989) (P values are based on 1,000 simulated samples), as well as Harpending's r (Harpending et al. 1993), as implemented in ARLEQUIN 2.0. Both Fu's F_s and Tajima's D were not significantly different from zero ($F_s = -0.66$, $P = .35$; $D = -0.19$, $P = .50$), suggesting no demographic expansion of the Andamanese population. In addition, Harpending's r value was 0.146, consistent with a scenario of constant population size.

Assuming a constant population size, the Kimura two-parameter model of substitution (as implemented in MEGA 2.1) and a mutation rate of 1.65×10^{-7} per site per year (corresponding to 33% divergence per million years [Ward et al. 1991]), divergence times of the Andaman M2 haplotypes relative to two different founders were estimated. When the most ancestral Andaman-specific M2 type (bearing the motif 16223T, 16319A, 16357C) (fig. 1) was used as a founder, the average time needed to accumulate the observed variation in Andaman Islands was 25,300 years. When the Indian candidate as ancestral type of the Andaman M2 sequences (bearing the motif 16223T, 16319A) (fig. 1) was used as a founder, the average time back to the Indian common ancestor was 42,300 years. These results indicate that Andaman islanders may have remained isolated from mainland India for 25,000–42,000 years. However, this time is almost certainly overestimated. The average substitution rate of the five substitutions found in the Andaman M2 cluster (16213, 16234, 16311, 16344, 16357)

(fig. 1) is ~2.5 times that of the control region as a whole (Meyer et al. 1999); thus, the real time to accumulate the observed variation may be up to 2.5-fold less, or 10,100–16,900 years. Moreover, the time estimates are based on the assumption that all the Andaman M2 types are derived from a single founding lineage; more recent times would result if there was more than one founding lineage. In summary, the presence of the unique M2 lineages in the Andaman Islands (as well as the Andaman M4 lineage that is identical to present-day Indian M4 lineages) suggests a colonization event from the Indian subcontinent occurring during the Upper Paleolithic (or perhaps even more recently), at most 40,000 years ago, which is still at least 20,000 years after the early southern-route migration to Sahul.

Thangaraj et al. (2003) also presented Y-chromosome evidence for Andaman islanders. They suggested that haplogroup D, an Asian-specific YAP lineage bearing the derived state at M174 (Underhill et al. 2000), is an original paternal lineage of Andaman islanders. To estimate the age of M174, we calculated that the average number of mutations from M174 to contemporary haplotypes was 4.89, on the basis of the data in Underhill et al. (2000). Underhill et al. (2000) estimated that the average time for a new mutation was 5,600–6,900 years, which puts the age of M174 at 27,000–34,000 years. Since the age of M174 is an upper estimate for the time when this Y haplogroup reached the Andaman Islands, the Y-chromosome evidence suggests an upper figure of ~30,000 years for the colonization of Andaman Islands, in good agreement with the mtDNA evidence. Hence, both Y-chromosome and mtDNA data support an Upper Paleolithic rather than Middle Paleolithic settlement of the archipelago.

Other studies have shown that mtDNA haplogroups M and U are common in the Indian subcontinent, and both have coalescence times of 50,000–70,000 years (Quintana-Murci et al. 1999; Kivisild et al. 1999*a*, 1999*b*, 2003). This has been taken as a genetic signature for an "early" (i.e., Middle Paleolithic) colonization of South Asia by modern humans and, consequently, as a confirmation of the "southern route" hypothesis. However, in South Asia, the earliest evidence of modern human industries and remains is dated to ~30,000 years (Kennedy and Deraniyagala 1989; Misra 2001). Because earlier records from Neanderthal-like technologies are found in South Asia (Misra 2001), the absence of modern human traces does not seem to be due to a gap in the archeological record. How to explain a gap of at least 20,000 years long between the archeological and genetic evidence? The coalescence time of haplogroup U in Europe is also dated to 50,000–60,000 years (Richards et al. 2000), but the accepted time scale of the peopling of Europe by modern humans is 35,000–40,000 years ago (Bocquet-Appel and Demars 2000). If so, why should South Asia, but not Europe, be considered to have been colonized 50,000–70,000 years ago? The observed timing discrepancies between genetic and archeological estimates are simply explained by the fact that the divergence of genes predates that of populations (Barbujani et al. 1998). Consequently, the evidence for a Middle Paleolithic colonization of the Indian subcontinent provided by mtDNA studies and the genetic confirmation of the "southern route" hypothesis are all the more debatable.

What conclusive genetic evidence remains for the "southern route" hypothesis? If the Indian subcontinent had received migrants from this putative migration from Africa to the Sahul, it would be reasonable to expect at least some remote affinities between African, Indian, Australian, and/or Papua-New-Guinean (PNG) gene pools. Unfortunately, to date, most studies aimed at detecting traces of an ancient peopling of South Asia through the southern route have not compared the Indian data in a wider context, in particular with people (except Africans) from regions believed to have been involved in the "southern route" migration. *Alu* insertions data are interpreted as supporting an ancient African-PNG relationship, but India is not a part of this relationship (Stoneking et al. 1997). Y-chromosome and mtDNA data suggest a connection between the Indian subcontinent and Australia, which is, however, dated to <5,000 years (Redd and Stoneking 1999; Redd et al. 2002; Cordaux et al. 2003). Thangaraj et al. (2003) suggest a possible common origin for the inhabitants of the Andaman Islands and PNG, on the basis of high frequencies of mtDNA 16357C in these two areas only. However, we note that 16357C in Andamanese mtDNAs occurs on the background of 16223T and 16319A, corresponding to haplogroup M (Endicott et al. 2003; Thangaraj et al. 2003). In contrast, 16357C in PNG is associated with 16223C and 16319G (Hagelberg et al. 1999; Redd and Stoneking 1999), corresponding to haplogroup N. Consequently, the presence of 16357C in the Andaman Islands and PNG is most likely the result of parallel mutations and not a signature of shared ancestry. In addition, the putative original paternal lineage of Andaman islanders (i.e., Y haplogroup D) (Thangaraj et al. 2003) is not found in Melanesia or Australia (Kayser et al. 2003). Otherwise, mtDNA haplotypes in South Asian ethnic groups are most closely related to east Eurasians and do not show any particular ties to African or PNG populations (Kivisild et al. 2003; Cordaux et al. 2003). In addition, an mtDNA control region motif proposed by Forster et al. (2001) to represent a signature of an early migration from Africa to Sahul through the southern route is not found in South Asia (Cordaux et al. 2003). In summary, there is no convincing support to date for a Middle Paleolithic genetic contribution to

South Asia by migrants from Africa to Sahul along the southern route.

If so, and in light of the genetic and archeological evidence, the most reasonable scenario for the peopling of South Asia is an Upper Paleolithic event (i.e., the major expansion of modern humans out of Africa through the Levant [Lahr and Foley 1994]), from which the current Indian gene pool is derived. Proto-Eurasians subsequently evolved to their present distinct South Asian, East Asian, and European gene pools and expanded ~30,000 years ago (Forster et al. 2001). Without requiring a Middle Paleolithic migration of modern humans into South Asia, this scenario explains why (i) most South Asian mtDNA clusters coalesce and show signs of demographic expansions ~30,000 years ago (Kivisild et al. 1999*b*), (ii) the South Asian mtDNA gene pool is related to (but distinct from) other Eurasian mtDNA pools, (iii) the South Asian mtDNA gene pool does not show close affinities to either Africa or PNG, and (iv) the archeological record does not show evidence for the presence of modern humans in South Asia before ~30,000 years ago. Hypothesizing a Middle Paleolithic migration to South Asia would create more problems than it would solve: it would, in particular, hardly explain the above crucial points iii and iv.

We conclude that there is currently no convincing genetic evidence that supports the postulated Middle Paleolithic migration of modern humans from Africa to the Sahul through South Asia. This does not necessarily mean that such a migration never occurred, since archaeological evidence does document modern humans in Sahul by ~60,000 years ago. However, it is possible that subsequent Upper Paleolithic migrations in Eurasia erased the genetic traces in contemporary populations of this early event in our history (Cavalli-Sforza et al. 1994; Cordaux et al. 2003); in any event, the "southern route" hypothesis still awaits genetic support.

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Electronic-Database Information

URLs for data presented herein are as follows:

ARLEQUIN 2.0, http://lgb.unige.ch/arlequin/
MEGA 2.1, http://www.megasoftware.net/

References

Anderson S, Bankier AT, Barrell BG, De Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich D, Roe B, Sanger F, Schreier P, Smith A, Staden R, Young I (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Bamshad M, Kivisild T, Watkins WS, Dixon ME, Ricker CE, Rao BB, Naidu JM, Prasad BVR, Reddy PG, Rasanayagam A, Papiha SS, Villems R, Redd AJ, Hammer MF, Nguyen SV, Carroll ML, Batzer MA, Jorde LB (2001) Genetic evidence on the origins of Indian caste populations. Genome Res 11: 994–1004

Barbujani G, Bertorelle, G, Chikhi L (1998) Evidence for Paleolithic and Neolithic gene flow in Europe. Am J Hum Genet 62:488–491

Bocquet-Appel JP, Demars PY (2000) Neanderthal contraction and modern human colonization of Europe. Antiquity 74: 544–552

Cavalli-Sforza LL, Menozzi P, Piazza A (1994) History and geography of human genes. Princeton University Press, Princeton, NJ

Cordaux R, Saha N, Bentley GR, Aunger R, Sirajuddin SM, Stoneking M (2003) Mitochondrial DNA analysis reveals diverse histories of tribal populations from India. Eur J Hum Genet 11:253-264

Endicott P, Gilbert MTP, Stringer C, Lalueza-Fox C, Willerslev E, Hansen AJ, Cooper A (2003) The genetic origins of Andaman Islanders. Am J Hum Genet 72:178–184

Forster P, Torroni A, Renfrew C, Röhl A (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. Mol Biol Evol 18:1864–1881

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

Hagelberg E, Goldman N, Lio P, Whelan S, Schiefenhövel W, Clegg JB, Bowden DK (1999) Evidence for mitochondrial DNA recombination in a human population of island Melanesia. Proc R Soc Lond Biol Sci 266:485–492

Harpending HC, Sherry ST, Rogers AR, Stoneking M (1993) The genetic structure of ancient human populations. Curr Anthropol 34:483–496

Johnson BJ, Miller GH, Fogel ML, Magee JW, Gagan MK, Chivas AR (1999) 65,000 years of vegetation change in central Australia summer monsoon. Science 284:1150–1152

Kayser M, Brauer S, Weiss G, Schiefenhövel W, Underhill PA, Shen P, Oefner P, Tommaseo-Ponzetta M, Stoneking M (2003) Reduced Y-chromosome, but not mitochondrial DNA, diversity in human populations from West New Guinea. Am J Hum Genet 72:281–302

Kennedy KAR, Deraniyagala SU (1989) Fossil remains of 28,000-year-old hominids from Sri Lanka. Curr Anthropol 30:394–399

Kivisild T, Bamshad MJ, Kaldma K, Metspalu E, Reidla M, Laos S, Parik J, Watkins WS, Dixon ME, Papiha SS, Mastana SS, Mir MR, Ferak V, Villems R (1999*a*) Deep common ancestry of Indian and western-Eurasian mitochondrial DNA lineages. Curr Biol 9:1331–1334

Kivisild T, Kaldma K, Metspalu M, Parik J, Papiha S, Villems R (1999*b*) The place of the Indian mitochondrial variants

in the global network of maternal lineages and the peopling of the old world. In: Papiha SS, Deka R, Chakraborty R (eds) Genomic diversity: applications in human population genetics. Kluwer Academic/Plenum Publishers, New York, pp 135–152

Kivisild T, Rootsi S, Metspalu M, Mastana S, Kaldma K, Parik J, Metspalu E, Adojaan M, Tolk H-V, Stepanov V, Gölge M, Usanga E, Papiha SS, Cinnioğlu C, King R, Cavalli-Sforza L, Underhill PA, Villems R (2003) The genetic heritage of the earliest settlers persists both in Indian tribal and caste populations. Am J Hum Genet 72:313–332

Lahr MM, Foley R (1994) Multiple dispersals and modern humans origins. Evol Anthropol 3:48–60

Meyer S, Weiss G, von Haeseler A (1999) Pattern of nucleotide substitution and rate heterogeneity in the hypervariable regions I and II of human mtDNA. Genetics 152:1103–1110

Miller GH, Magee JW, Johnson BJ, Fogel ML, Spooner NA, McCulloch MT, Ayliffe LK (1999) Pleistocene extinction of *Genyornis newtoni*: human impact on Australian megafauna. Science 283:205–208

Misra VN (2001) Prehistoric human colonization of India. J Biosci 26:491–531

Quintana-Murci L, Semino O, Bandelt H-J, Passarino G, McElreavey K, Santachiara-Benerecetti AS (1999) Genetic evidence on an early exit of *Homo sapiens sapiens* from Africa through eastern Africa. Nat Genet 23:437–441

Redd AJ, Roberts-Thomson J, Karafet T, Bamshad M, Jorde LB, Naidu JM, Walsh B and Hammer MF (2002) Gene flow from the Indian subcontinent to Australia: evidence from the Y chromosome. Curr Biol 12:673–677

Redd AJ, Stoneking M (1999) Peopling of Sahul: mtDNA variation in Aboriginal Australian and Papua New Guinean populations. Am J Hum Genet 65:808–828

Richards M, Macaulay V, Hickey E, Vega E, Sykes B, Guida V, Rengo C, et al (2000) Tracing European founder lineages in the Near Eastern mtDNA pool. Am J Hum Genet 67: 1251–1276

Roberts RG, Jones R (1994) Luminescence dating of sediments: new light on the human colonization of Australia. Australian Aboriginal Studies 2:2–17

Stoneking M, Fontius JJ, Clifford SL, Soodyall H, Arcot SS, Saha N, Jenkins T, Tahir MA, Deininger PL, Batzer MA (1997) Alu insertion polymorphisms and human evolution: evidence for a larger population size in Africa. Genome Res 7:1061–1071

Stringer CB, Andrews P (1988) Genetic and fossil evidence for the origin of modern humans. Science 239:1263–1268

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Thangaraj K, Singh L, Reddy AG, Rao VR, Sehgal SC, Underhill PA, Pierson M, Frame IG, Hagelberg E (2003) Genetic affinities of the Andaman Islanders, a vanishing human population. Curr Biol 13:86–93

Thorne A, Grün R, Mortimer G, Spooner NA, Simpson JJ, McCulloch M, Taylor L, Curnoe D (1999) Australia's oldest human remains: age of the Lake Mungo 3 skeleton. J Hum Evol 36:591–612

Underhill PA, Shen P, Lin AA, Jin L, Passarino G, Yang WH, Kauffman E, Bonné-Tamir B, Bertranpetit J, Francalacci P, Ibrahim M, Jenkins T, Kidd JR, Mehdi SQ, Seielstad MT, Spencer Wells R, Piazza A, Davis RW, Feldman MW, Cavalli-Sforza LL, Oefner PJ (2000) Y chromosome sequence variation and the history of human populations. Nat Genet 26: 358–361

Ward RH, Frazier BL, Dew-Jager K, Pääbo S (1991) Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720–8724

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Reply to Cordaux and Stoneking

To the Editor:

Cordaux and Stoneking (2003 [in this issue]) have argued that although there is evidence of anatomically modern humans (AMH) reaching Australia ~60,000 years ago (see also Bowler et al. 2003), there is no convincing genetic evidence that these early migrants passed through South Asia. They see this as obviating the need for a "southern migration" route for AMH out of Africa. They suggest that the ancestors of all known non-African mtDNA lineages (haplogroups M and N) entered Eurasia via the Levant ~45,000 years ago, prior to differentiating and dispersing to all land areas outside of Africa. Cordaux and Stoneking also seek to use estimated dates for the colonization of the Andaman Islands to lend support to their position.

The "crucial points" of evidence cited by Cordaux and Stoneking for their view on the settlement of South Asia are that "the South Asian mtDNA gene pool does not show close affinities to either Africa or PNG" (Papua New Guinea), and that "the archeological record does not show evidence for the presence of modern humans in South Asia before ~30,000 years ago." This position is inconsistent, since they first equate "modern" humans with Middle Paleolithic tools in Australia at ~50,000 years ago (Bowler et al. 2003) but later exclude South Asian settlers within the same time frame from being "modern" on the basis of their lithic industries. Here we re-evaluate the evidence for this position and show that the distributions and characteristics of mtDNA and Y-chromosome data actually accord better with a dispersal of AMH into South Asia from Africa >30,000 years ago. The same data also argue for the hypothesized "southern route" "out of Africa," leaving through Ethiopia via Arabia.

We agree with Cordaux and Stoneking that the diversity within Andamanese M2 is potentially useful for giving some idea of the settlement time for the Andaman Islands, because it is plausible that a regionally specific clade in the mtDNA genealogy (in this case, Andamanese M2) had its most recent common ancestor (MRCA) in that region. Of course, it is possible that related mtDNAs in the source population may have disappeared because of drift, but, in that case, the MRCA of the cluster in the settled region could either pre- or postdate the colonization event (for a careful discussion of the issues, see Richards et al. [2000]). An estimate of the time to the MRCA of the clade, with the available Andamanese data, provides an idea of the *latest* possible settlement time and not the earliest (contra Barbujani 1998 and Cordaux and Stoneking 2003 [in this issue]).

There are further statistical problems in the reply by Cordaux and Stoneking. In trying to justify a constant population size model for the Andamanese, they concentrate their calculations on a set of lineages *chosen to fall within a particular clade,* whereas the usual theory pertains to random sequences from the whole population, which, in the case of the Andamanese, contains the M4 clade. Thus, it is not clear what the sampling distributions of the test statistics under the null hypothesis would be for their approach. Therefore, it is not possible to assess the significance of the values computed. Further, their assertion that the point estimates of divergence are "almost certainly overestimated" because the varied sites are faster mutating than average is based on a misunderstanding of how mutations arise. The number of mutations accumulates proportionally to the *average* rate for the whole DNA stretch under consideration, irrespective of the individual site rates. Of course, some sites may be hit more than once, but this effect would lead to an *underestimation* of divergence times.

Cordaux and Stoneking introduce the finding by Thangaraj et al. (2003) that the present-day indigenous Andamanese belong to Y-chromosome haplogroup D, defined by the mutation M174. Although the date of haplogroup D is not incompatible with the Upper Paleolithic, they assume that both mtDNA and Y chromosome yield similar narratives regarding archeologically defined horizons. However, sex-specific processes may lead to more genetic drift in males than in females, so that the time depth of the Y genealogy would be more recent. Consequently, the apparently discordant Upper and Middle Paleolithic molecular dates, for Y and mtDNA, respectively, would not necessarily be incompatible.

There are other reasons that the estimated date to the MRCA of the M174 Y-chromosome clade (27,000–34,000 years) of Cordaux and Stoneking requires clarification. (i) The authors have not provided CIs for their estimate from a sample of just nine M174 individuals from two derived clades, and so the dates given only reflect uncertainty in the mutation rate. (ii) The Y SNP data sets used suffer from strong ascertainment bias (Underhill et al. 2000). Therefore, dating methods applicable to randomly selected mutations are likely to be seriously biased when applied to such data.

Using the subset of 42 unambiguous Andamanese mtDNA sequences available (Thangaraj et al. 2003) and considering the region 16090–16365 (Andrews et al. 1999) for which a plausible average mutation rate estimate is available (Forster et al. 1996), we estimate the time to the MRCA of the clade with motif 16223/16319/16357 as 33,000 years (SE 22,000) and to 16223/16319 as 53,000 years (SE 30,000). This method is based on the average number of mutations of each sequence from the ancestral one and an estimate of its SE (Saillard et al. 2000).

Given the large SEs of these estimates, the statement of Cordaux and Stoneking that this "suggests a colonization event…at most 40,000 years ago" cannot be sustained. In fact, the diversity of Andamanese M2 allows us to pin it down only to the last 100,000 years, but it must necessarily postdate the MRCA of haplogroup M, which is 65,000 years old (SE 7,000), on the basis of a recent dating from complete mtDNA sequences (Mishmar et al. 2003). The current estimate of coalescence for M2, on the basis of HVS-I variation, of 73,000 years (SE 22,900) does not further restrict its age (Kivisild et al. 2003).

Cordaux and Stoneking place their estimates of the first appearance of haplogroup M in South Asia within the context of a "southern route" of dispersal for AMH from Africa to Oceania. This hypothesis is directly linked to the absence of AMH in the Levantine corridor at the time of their putative presence in Australia ~60,000 years ago. On the other hand, Endicott et al. (2003) said "that haplogroup M may represent the phylogenetic signature of an early, southern colonization route in Asia." Neither the references for this statement nor the authors invoked an early settlement of Australia (contra Cordaux and Stoneking) to explain an African exodus leaving by a southern route (i.e., from Ethiopia via Arabia). Rather, it is the distribution and estimates of coalescence for mtDNA haplogroup M that are the basis for suggesting its early presence in South Asia.

All non-Africans have inherited a subset of African mtDNA haplogroup L3 lineages, differentiated into haplogroups M and N. The lack of other L3 lineages among all non-African mitochondria suggests that the earliest migration(s) of AMH already carried those two mtDNA ancestors or that they have replaced previously extant lineages. Haplogroup M is present in Ethiopia, but the rest of M is densely distributed throughout South and East Asia and is absent in the Near East and Europe. If the settlement of Asia by haplogroup M–bearing populations was delayed by the ~30,000 years alluded to

by Cordaux and Stoneking, there is, as yet, no evidence of the source population, either inside or outside of Africa. Since haplogroup M is not present along their hypothesized "northern route" "out-of-Africa," it seems likely to have been dispersed along the "southern route" and necessarily "early," since the MRCA of Asian M is ~65,000 years old (Quintana-Murci et al. 1999; Stringer 2000; Mishmar et al. 2003).

The distributions, diversity, and ages of haplogroups M and N in India are consistent with this region providing the inocula for the subsequent differentiation of both the distinctive eastern and western Eurasian gene pools (Kivisild et al. 2003). This is because (i) the percentages of typically eastern and western Eurasian mtDNA lineages present in India are moderately low, (ii) the majority of these lineages are specific to India, and (iii) they contain all the founding haplotypes for the non-African mtDNA gene pool. The time to coalescence of the major M subclusters on the Indian subcontinent center on 47,000 years (SE 2,500 years) and not the ~30,000 years that Cordaux and Stoneking (2003 [in this issue]) allude to (Kivisild et al. 1999). These Indian components are comparable in diversity and are older than most eastern Asian and Papuan haplogroup M clusters (Forster et al. 2001). This suggests that the Indian subcontinent was settled soon after the African exodus and that there has been no complete extinction, or replacement, of the initial settlers (Kivisild et al. 2003). Of the Indian haplogroup M lineages, M2 is significant because of its estimated age (73,000 years, SE 20,000), southerly distribution, and frequency (~10% of caste populations and ~23% of noncaste populations) (Kivisild et al. 1999; Bamshad et al. 2001; Endicott et al. 2003).

The phylogeography of the four non-African Y-chromosomal founder haplogroups C, D, F, and K is also more consistent with the southern route migration than one through the Levantine corridor. This is because (i) the presence of the D lineage in the Andaman Islands completes the package of the postulated founder lineages detected in or around India, and (ii) C and D are spread, like mitochondrial haplogroup M, only in the Asian continent and Oceania (Underhill et al. 2001; Kivisild et al. 2003) and not in western Eurasia and North Africa. Further, the upper limit (contra Cordaux and Stoneking 2003 [in this issue]) for the time of their spread out of Africa would be the coalescent of all three main branches and not of only one of them, D-M174.

On the basis of the mtDNA and Y-chromosome data presented here, we see no need to accept the view of Cordaux and Stoneking regarding the settlement of South Asia. To suggest that in this region "the archeological record does not show evidence for the presence of modern humans…before ~30,000 years ago" is not sustainable if they accept their presence in Australia at ~60,000 years ago. This Eurocentric view of equating AMH with Upper Paleolithic tools has already been abandoned in the Middle East and Africa (McBrearty and Brooks 2000) and, given the Australasian evidence, should be discarded in South Asia, too. We must infer an early dispersal of AMH with non–Upper Paleolithic technology through Asia to explain the early Australian evidence (Stringer 2000), although we agree with Cordaux and Stoneking that the precise route(s) taken is still unclear (Stringer 2002). But we see no requirement for the South Asian mtDNA gene pool to demonstrate close affinities with either PNG or Africa to discuss an early settlement of this region. Given the continuity of the archeological record within India, from the Middle Paleolithic onward, and the range of estimated dates for Indian haplogroup M, there is no clear reason to preclude the presence of modern humans in this region prior to ~30,000 years ago.

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References

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147

Bamshad M, Kivisild T, Watkins S, Dixon M, Ricker C, Rao B, Naidu J, Prasad B, Reddy P, Rasanayagam A, Papiha S, Villems R, Redd A, Hammer M, Nguyen S, Carroll M, Batzer M, Jorde L (2001) Genetic evidence on the origins of Indian caste populations. Genome Res 11:994–1004

Barbujani G, Bertorelle G, Chikhi L (1998) Evidence for Paleolithic and Neolithic gene flow in Europe. Am J Hum Genet 62:488–491

Bowler JM, Johnston H, Olley JM, Prescott JR, Roberts RG, Shawcross W, Spooner NA (2003) New evidence for human occupation and climatic change at Lake Mungo, Australia. Nature 421:837–840

Cordaux R, Stoneking M (2003) South Asia, the Andamanese and the genetic evidence for an "early" human dispersal out of Africa. Am J Hum Genet 72:1586–1590 (in this issue)

Endicott P, Gilbert MTP, Stringer C, Lalueza-Fox C, Willerslev

E, Hansen AJ, Cooper A (2003) The genetic origins of the Andaman Islanders. Am J Hum Genet 72:178–184
Forster P, Harding R, Torroni A, Bandelt H-J (1996) Origin and evolution of Native American mtDNA variation: a reappraisal. Am J Hum Genet 59:935–945
Forster P, Torroni A, Renfrew C, Rohl A (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. Mol Biol Evol 18:1864–1881
Kivisild T, Kaldma K, Metspalu M, Parik J, Papiha SS, Villems R (1999) The place of the Indian mitochondrial DNA variants in the global network of maternal lineages and the peopling of the old world. In: Papiha SS, Deka R, Chakraborty R (eds) Genomic diversity: applications in human population genetics. Kluwer/Academic/Plenum, New York, pp 135–152
Kivisild T, Rootsi S, Metspalu M, Mastana S, Kaldma K, Parik J, Metspalu E, Adojaan M, Tolk H-V, Stepanov V, Gölge M, Usanga E, Papiha SS, Cinnioğlu C, King R, Cavalli-Sforza L, Underhill PA, Villems R (2003) The genetic heritage of the earliest settlers persists in both the Indian tribal and caste populations. Am J Hum Genet 72:313–332
McBrearty S, Brooks A (2000) The revolution that wasn't: a new interpretation of the origin of modern human behaviour. J Hum Evol 39:453–563
Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, Sukernik RI, Olckers A, Wallace DC (2003) Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Sci USA 100:171–176
Quintana-Murci L, Semino O, Bandelt H-J, Passarino G, McElreavey K, Santachiara-Benerecetti AS (1999) Genetic evidence of an early exit of *Homo sapiens sapiens* from Africa through eastern Africa. Nat Genet 23:437–441
Richards M, Macaulay V, Hickey E, Vega E, Sykes B, Guida V, Rengo C, et al (2000) Tracing European founder lineages in the Near Eastern mtDNA pool. Am J Hum Genet 67: 1251–1276
Saillard J, Forster P, Lynnerup N, Bandelt H-J, Nørby S (2000) mtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. Am J Hum Genet 67:718–726
Stringer C (2000) Coasting out of Africa. Nature 405:24–27
——— (2002) Modern human origins: progress and prospects. Philos Trans R Soc Lond B Biol Sci 357:563–579
Thangaraj K, Singh L, Reddy AG, Rao VR, Sehgal SC, Underhil PA, Pierson M, Frame IG, Hagelberg E (2003) Genetic affinities of the Andaman islanders, a vanishing population. Curr Biol 13:86–93
Underhill PA, Passarino G, Lin AA, Shen P, Lahr MM, Foley RA, Oefner PJ, Cavalli-Sforza LL (2001) The phylogeography of Y chromosome binary haplotypes and the origins of modern human populations. Ann Hum Genet 65:43–62
Underhill PA, Shen P, Lin AA, Jin L, Passarino G, Yang WH, Kauffman E, Bonné-Tamir B, Bertranpetit J, Francalacci P, Ibrahim M, Jenkins T, Kidd JR, Medhi SQ, Seielstad MT, Wells RS, Piazza A, Davis RW, Feldman MW, Cavalli-Sforza LL, Oefner PJ (2000) Y chromosome sequence variation and the history of human populations. Nat Genet 26:358–361

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Single-Nucleotide Polymorphisms and Glaucoma Severity

To the Editor:

A recent publication in the *Journal* (Copin et al. 2002) reported that SNPs in the promoter of apolipoprotein E (APOE [MIM 107741]) modify the phenotype of primary open-angle glaucoma, result in increased optic-nerve damage, and interact at a highly significant level with an SNP in the promoter of myocilin (MYOC [MIM 601652]), a known glaucoma-causing gene. If correct, this would be of considerable importance for providing novel insight into the pathogenesis of a leading cause of worldwide blindness (Quigley et al. 1993), which is characterized by visual-field loss and progressive excavation (cupping) of the optic disc.

That study's conclusions are entirely dependent on the observation of differing disease severity in the genotypic subgroups. Glaucoma severity was graded by use of ordinal scales, and it is important to relate statistical analysis back to these scales. If we consider a simple example of patients with a bacterial infection that is scored (1, 2, or 3) according to whether they "got better," "stayed the same," or "got worse," if equal numbers got better and got worse, it would be meaningless to state that, on average, patients stayed the same (Campbell 2001). It would be equally invalid to present fractional differences in the data (e.g., 1.3). Unfortunately, Copin et al. (2002) employed this approach with both parameters used to gauge glaucoma severity.

The first parameter, cup-to-disc ratio (CDR), estimates in 10% (0.1) increments the proportion of the optic nerve that has been damaged. CDR is only an approximate guide because of high interindividual (normal range 0.0 to almost 0.9) and interobserver variability (>0.2) among specialists assessing optic discs (Lichter 1976; Jonas et al. 1988; Tielsch et al. 1988). Although CDR is a form of ordinal data with a fixed scale (0.1, 0.2, 0.3, etc.), Copin and coworkers (2002) report fractional differences (0.03 or 0.06), smaller than the scale increments, as evidence of increased disease severity with particular genotypes. The second parameter, visual-field loss, has been similarly evaluated. Recorded with an unspecified number of different techniques, the data were reanalyzed with a version

of the authors' semiquantitative five-point scale that differs from the one cited (Brézin et al. 1997) and that does not appear to have been prospectively evaluated relative to more widely used grading systems (Advanced Glaucoma Intervention Study 1994). Again, it is unclear how a fractional difference (mean 0.6) in a narrow, whole-integer scale (2 = early defect; 3 = moderate [arcuate] defect; 4 = advanced defect) can be interpreted.

Without supportive clinical data, evidence is lacking that APOE SNPs either are associated with a more severe phenotype or interact at a highly significant level with an SNP in the MYOC promoter. Since a large prospective study (Alward et al. 2002) failed to replicate the authors' report of an association between the MYOC promoter SNP and glaucoma severity (Colomb et al. 2001), the hypotheses that either APOE or MYOC promoter SNPs affect the severity of glaucoma (Copin et al. 2002), for now, remain to be proven.

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Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for APOE and MYOC)

References

Advanced Glaucoma Intervention Study (1994) Visual field test scoring and reliability. Ophthalmology 101:1445–1455

Alward WLM, Kwon YH, Khanna CL, Johnson T, Hayreh SS, Zimmerman MB, Narkiewicz J, Andorf JL, Moore PA, Fingert JH, Sheffield VC, Stone EM (2002) Variations in the myocilin gene in patients with open-angle glaucoma. Arch Ophthalmol 120:1189–1197

Brézin AP, Bechetoille A, Hamard P, Valtot F, Berkani M, Belmouden A, Adam MF, Dupont de Dinechin S, Bach JF, Garchon HJ (1997) Genetic heterogeneity of primary open angle glaucoma and ocular hypertension: linkage to GLC1A associated with an increased risk of severe glaucomatous optic neuropathy. J Med Genet 34:546–552

Campbell MJ (2001) Statistics at square two. BMJ Publishing, London

Colomb E, Nguyen TD, Béchetoille A, Dascotte J-C, Valtot F, Brézin AP, Berkani M, Copin B, Gomez L, Polansky JR, Garchon H-J (2001) Association of a single nucleotide polymorphism in the TIGR/MYOCILIN gene promoter with the severity of primary open-angle glaucoma. Clin Genet 60:220–225

Copin B, Brézin AP, Valtot F, Dascotte J-C, Béchetoille A, Garchon H-J (2002) Apolipoprotein E–promoter single-nucleotide polymorphisms affect the phenotype of primary open-angle glaucoma and demonstrate interaction with the myocilin gene. Am J Hum Genet 70:1575–1581

Jonas JB, Gusek GC, Naumann GO (1988) Optic disc, cup and neuroretinal rim size, configuration and correlations in normal eyes. Invest Ophthalmol Vis Sci 29:1151–1158

Lichter PR (1976) Variability of expert observers in evaluating the optic disc. Trans Am Ophthalmol Soc 74:532–572

Quigley HA (1993) Open-angle glaucoma. N Engl J Med 328: 1097–1106

Tielsch JM, Katz J, Quigley HA, Miller NR, Sommer A (1988) Intraobserver and interobserver agreement in measurement of optic disc characteristics. Ophthalmology 95:350–356

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Reply to Bunce et al.

To the Editor:

Bunce et al. (2003 [in this issue]) did not question the validity of the statistical method, nonparametric, that was used for testing an explanatory potential of apolipoprotein E (APOE) genotypes relative to glaucoma phenotype variation. Nor did they criticize the second part of our study, which was relative to an influence of APOE polymorphism on intraocular pressure.

Their comment regarding an ordinal nature of the cup-to-disk ratio is unexpected, as the cup-to-disk ratio—the ratio of the diameters of the excavation and of the optic disc—is fractional by definition.

This measure of the optic-nerve status remains commonly used by clinicians and researchers, especially in the area of glaucoma genetics (Alward et al. 2002). It is reassuring to read a recent article contributed by three of the authors of this letter (Aung et al 2003) that uses it, with values taken between the increments (table 2 of the article).

Contrary to the statement of Bunce et al., the scale that we used for grading the visual-field loss was similar to that described elsewhere (Brézin et al. 1997). Critical for the consistency of our data set, cup/disc ratios and visual-field evaluations were tightly correlated (nonparametric correlations: Spearman R 0.596, $P < 1 \times 10^{-17}$; Kendall τ 0.496, $P < 1 \times 10^{-8}$; γ 0.625, $P < 1 \times 10^{-8}$).

The interesting study of Alward et al. (2002) was clearly not prospective, and it did not investigate a role of APOE. A detailed discussion of the reasons for the discordance

between this study and that of Colomb et al. (2001) goes beyond the scope of this reply letter and will be given elsewhere. In brief, however, there were substantial differences between the patient samples, notably the age at diagnosis. Investigating these differences will provide insight into the significance of the SNPs for glaucoma phenotypes.

Although negative results may be most instructive, it is also essential to obtain data that are supportive of an initial finding. The readers of the *Journal* should be aware of two recent reports that are consistent with an involvement of APOE in glaucoma (Bayer et al. 2002; Vickers et al. 2002).

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References

Alward WL, Kwon YH, Khanna CL, Johnson AT, Hayreh SS, Zimmerman MB, Narkiewicz J, Andorf JL, Moore PA, Fingert JH, Sheffield VC, Stone EM (2002) Variations in the myocilin gene in patients with open-angle glaucoma. Arch Ophthalmol 120:1189–1197

Aung T, Okada K, Poinoosawmy D, Membrey L, Brice G, Child AH, Bhattacharya SS, Lehmann OJ, Garway-Heath DF, Hitchings RA (2003) The phenotype of normal tension glaucoma patients with and without OPA1 polymorphisms. Br J Ophthalmol 87:149–152

Bayer AU, Keller ON, Ferrari F, Maag KP (2002) Association of glaucoma with neurodegenerative diseases with apoptotic cell death: Alzheimer's disease and Parkinson's disease. Am J Ophthalmol 133:135–137

Brézin AP, Bechetoille A, Hamard P, Valtot F, Berkani M, Belmouden A, Adam MF, Dupont de Dinechin S, Bach JF, Garchon HJ (1997) Genetic heterogeneity of primary open angle glaucoma and ocular hypertension: linkage to GLC1A associated with an increased risk of severe glaucomatous optic neuropathy. J Med Genet 34:546–552

Bunce C, Hitchings RA, Bhattacharya SS, Lehmann OJ (2003) Single-nucleotide polymorphisms and glaucoma severity. Am J Hum Genet 72:1593–1594 (in this issue)

Colomb E, Nguyen TD, Béchetoille A, Dascotte J-C, Valtot F, Brézin AP, Berkani M, Copin B, Gomez L, Polansky JR, Garchon H-J (2001) Association of a single nucleotide polymorphism in the TIGR/MYOCILIN gene promoter with the severity of primary open-angle glaucoma. Clin Genet 60:220–225

Vickers JC, Craig JE, Stankovich J, McCormack GH, West AK, Dickinson JL, McCartney PJ, Coote MA, Healey DL, MacKey DA (2002) The apolipoprotein epsilon4 gene is associated with elevated risk of normal tension glaucoma. Mol Vis 8:389–393

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