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Address for correspondence and reprints: Marie-Laure Martin Negrier, Laboratoire d'Anatomie Pathologique, CHR Pellegrin, 33076 Bordeaux, Cedex, France. E-mail: mlmartin@hippocrate.u-bordeaux2.fr

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Reply to Inglehearn

To the Editor:

In our article “Localization of a Novel X-Linked Progressive Cone Dystrophy Gene to Xq27: Evidence for Genetic Heterogeneity” (Bergen and Pinckers 1997), we presented evidence favoring a location, on Xq27, for a cone dystrophy gene. This localization is questioned by Dr. Inglehearn (1998) in his letter “LOD Scores, Location Scores, and X-Linked Cone Dystrophy.” Although Dr. Inglehearn makes a good (methodological) point, we feel that the majority of his criticism is not justified.

Clearly, as Dr. Inglehearn states correctly, figure 2 in our previous article (Bergen and Pinckers 1997) shows a picture of the multipoint location scores rather than

of the multipoint LOD scores. Although the presentation of location scores instead of multipoint LOD scores is not wrong in itself, it is rather unconventional and therefore confusing. Thus, we agree that, with regard to a multipoint location score of 10.8, the calculated multipoint LOD score is indeed 2.35. Obviously, for X-chromosomal disorders, the latter score is still considered to be significant.

Subsequently, Dr. Inglehearn calculates, on the basis of the data presented, multipoint (maximum?) LOD scores of 3.38 and 2.46 at DXS998, using different LOD-score strategies. Unfortunately, additional calculations for other markers are not given. Both these LOD scores for DXS998 are higher than the true multipoint LOD scores calculated by us (maximum LOD score [Z_{max}] of 2.35). Thus, in our article (Bergen and Pinckers 1997), our calculation of LOD scores and our choice of parameters were in fact very conservative. Therefore, the assertion by Dr. Inglehearn (1998) that “these data do indeed suggest a locus for X-linked cone dystrophy in this region but with rather less significance than Bergen and Pinckers have stated” (p. 900) is not justified. Most likely, the true findings for the Z_{max} score at DXS998 are somewhere within the range 2.35–3.38.

Dr. Inglehearn states that a second weakness of the article is the order and placement of markers used in the multipoint linkage analysis. However, this assertion is based on out-of-date and incomplete genetic maps of the region, as indicated by the references to literature published in 1992 and 1994 (NIH/CEPH Collaborative Mapping Group 1992; Gyapay et al. 1994), and therefore is not justified. Much more recent and up-to-date consensus maps (Dib et al. 1996) place DXS998 ~15 cM from the distal tip of the X chromosome and at least 7 cM proximal to the red cone pigment (RCP)/green cone pigment (GCP) gene cluster.

In addition, in our article (Bergen and Pinckers 1997), data on two additional markers, DXS297 and DXS1123, are presented. Both DXS297 and DXS1123 reveal higher (maximum two-point) LOD scores of 2.54 and 2.60, respectively, without recombination with COD2, but these markers are ignored in the comments by Dr. Inglehearn. Most likely, on the basis of recombination counting, haplotype analysis, and marker-to-marker analysis, both DXS297 and DXS1123 are part of a cosegregating haplotype, together with DXS998 and COD2. Although DXS297 and DXS1123 are not present on the CEPH/Généthon consensus maps, at least two independent reports in the literature (Richards et al. 1991; Donnelly et al. 1994) place DXS297 proximal to the fragile X site, which is located on Xq27.3 (Dib et al. 1996). Similar, although somewhat weaker, evidence can be found for DXS1123. In contrast, the RCP/GCP gene cluster is located on Xq28. In conclusion, there is

convincing evidence that the marker order used by us in our previous study is the (most) correct one.

On the basis of the likelihood data only (also see above), sufficient evidence for the “most likely” presence of a COD2 locus on Xq27 already existed; however, special *additional* attention was given to markers surrounding the RCP/GCP locus on Xq28, in view of the relatively close genetic distance between COD2 and the RCP/GCP cluster. Thus, the markers that very closely flank (0.5 cM each) the RCP/GCP gene cluster—namely, DXS8103 and DXS8069—were used. Again, this information can be obtained easily by detailed study of recent genetic databases.

On the basis of haplotype analysis only, the involvement of RCP/GCP in this pedigree is very unlikely. Markers DXS8103 and DXS8069 are only 1 cM apart and cosegregate with markers DXS52 and DXS1113, without recombination in the pedigree, when the fewest number of recombination events are assumed (see fig. 1 in Bergen and Pinckers 1997). If the RCP/GCP gene cluster is involved in the X-linked progressive cone dystrophy (XLPCD) in this pedigree, a double recombination event would be assumed to have occurred between DXS8103 and DXS8069 (potentially revealed by the haplotype of individual III-13/16). From multiple studies reported in the literature and from our own segregation data of hundreds of families, we know that such double recombination events on such a short stretch of DNA are extremely rare and occur in <0.1% of cases. Theoretically, without consideration of genetic interference down-regulating recombination of closely linked loci, the “risk for a double recombination” could be calculated as follows: (the chance of the first recombination occurring in 1 cM) \times (the chance of the second recombination occurring in 1 cM) \times (the number of meiosis in which these recombinations potentially could occur). If we assume that, in our pedigree, these recombinations could have taken place in ~ 5 meioses, which is the number of female meiosis between the two larger branches of the pedigree, then the overall risk for a double recombination not detected by our DNA analysis would be $.01 \times 5 \times .01 \times 5 = .0025$, or 0.25%. If we consider “genetic interference,” this figure most likely drops to $\leq 0.1\%$, which is the figure given above. In conclusion, on the basis of haplotype data and risk calculations, the chance that RCP/RCG is involved in XLPCD in our pedigree is $\leq 1:1,000$.

Given the complete cosegregation of both DXS8103 and DXS8069 with both DXS1113 and DXS52, two-point LOD scores for the first two markers and XLPCD are similar to those for the last two markers, which are given in our previous article (Bergen and Pinckers 1997). At a recombination fraction (θ) of .00, the LOD scores were -3.40 (DXS8103) or -4.26 (DXS1113). For the same markers, Z_{\max} is reached at $\theta = .05$ (LOD score

1.22) and at $\theta = .10$ (LOD score 0.57), respectively. If equal distances between RCP/GCP and both DXS8103 and DXS8069 (0.005 cM each) are assumed, the LOD scores would be 0.46 (DXS8103) and -0.377 (DXS8069) more than those for RCP/GCP.

Similar low(er) or negative LOD values are obtained with multipoint linkage analysis, with different combinations of various markers and RCP/GCP, different parameters, and different LOD-score strategies. Given the fact that markers at the Xq27 cluster (DXS998, DXS1123, and DXS297) reach a multipoint Z_{\max} of ~ 2.5 , the markers at RCP/GCP should reach multipoint LOD scores of >1.5 in order to be significant, according to the so-called $Z_{\max} - 1$ LOD unit rule. Instead, Z_{\max} scores at the RCP/GCP cluster remained <0.5 . Thus, by statistical means, the involvement of the RCP/GCP cluster was excluded in this pedigree.

On the other hand, the involvement of a rare and spontaneous Xq27/Xq28 dislocation or abnormal duplication(s) of the RCP/GCP gene cluster (as have been described elsewhere) or of other rearrangements further away from the RCP/GCP cluster or even other genetic mechanisms involved in the XLPCD in this pedigree could not and cannot yet be excluded. To obtain initial evidence for the exclusion of these hypotheses, however, Southern analysis with RCP/GCP cDNA was performed, and no structural abnormalities were found. Although this data alone does not *exclude* the involvement of RCP/GCP, they *do* suggest that involvement of RCP/GCP is even less likely, when considered in the context of the evidence that we obtained earlier.

The authors welcome the suggestion by Dr. Inglehearn that mutations or rearrangements upstream of the RCP/GCP locus possibly could be implicated in this XLPCD family, although our data suggest that such a genomic abnormality must be very much further away than the 43 kb mentioned. In conclusion, although the possible involvement of (regulatory elements of) the RCP/GCP gene cluster in the described XLPCD pedigree certainly is worth further investigation, the evidence accumulated thus far suggests the presence of a separate and distinct XLPCD locus, on Xq27.

A. A. B. BERGEN¹ AND A. J. L. G. PINCKERS²

¹*The Netherlands Ophthalmic Research Institute, Amsterdam; and* ²*Department of Ophthalmology, University of Nijmegen, Nijmegen, The Netherlands*

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Address for correspondence and reprints: Dr. A. A. B. Bergen, P. O. Box 12141, 1100 AC Amsterdam, The Netherlands. E-mail: A.Bergen@ioi.knaw.nl
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mtDNA Suggests Polynesian Origins in Eastern Indonesia

To the Editor:

mtDNA evidence has previously been interpreted as providing strong support for a model of rapid expansion of the Polynesian peoples from a homeland in Taiwan or southern China ~6,000 years ago into the remote Pacific. Here, we argue that the evidence is consistent with an alternative view, namely, that the Polynesian expansion originated within the Indonesian archipelago.

Several studies have been published concerning the settlement of the remote Pacific that use the phylogeographic analysis of mtDNA, either large-scale sampling and control-region sequence analysis (Lum et al. 1994; Redd et al. 1995; Sykes et al. 1995) or sequence-specific oligonucleotide analysis (Melton et al. 1995). These have distinguished two main hypotheses concerning Polynesian origins. The first hypothesis, often referred to somewhat incongruously as the “express train to Polynesia” (Diamond 1988), was proposed by Bellwood (1991, 1997). This suggests that the Polynesians originated in a demic expansion of Austronesian-speaking agriculturalists from the southern China mainland, ~6,000 years ago, and spread successively to Taiwan, the Philippines, eastern Indonesia, and then Melanesia, reaching Fiji by ~3,500 years ago and radiating across the Pacific to fill the Polynesian triangle by ~1,000 years ago. They would

have absorbed and replaced the local hunter-gatherer populations in Southeast Asia, who would have been of Australo-Melanesian ancestry. The principal alternative view, argued by Terrell (1986), is that the Polynesians evolved locally in Melanesia or, at least, within the voyaging corridor between the mainland and the Solomon Islands, defined by Irwin (1992).

Melton et al. (1995) and Redd et al. (1995) analyzed the history of a COII/tRNA^{Lys} intergenic 9-bp deletion by means of a suite of characteristic control-region transitions at positions 16189, 16217, 16247, and 16261 of the first hypervariable segment (according to the Cambridge Reference Sequence; Anderson et al. 1981). They referred to this as the “Polynesian motif,” because of its high frequencies in Polynesia, despite its occurrence farther west (Hagelberg and Clegg 1993; Redd et al. 1995). They traced the origin of this motif to Taiwan and proposed that this represented the Polynesian homeland, in line with the Bellwood (1997) hypothesis, while acknowledging that the motif itself probably arose in eastern Indonesia. Sykes et al. (1995) agreed in tracing the origin of the motif to Taiwan but also pointed out that the lack of the motif in Taiwan, Borneo, and the Philippines might complicate the issue. In addition, they pointed out, along with Lum et al. (1994), that somewhat <5% of Polynesians had control-region sequences derived from Melanesia. Furthermore, Sykes et al. (1995) distinguished a third hypothesis, proposed by Heyerdahl (1950), suggesting that Polynesian ancestry may have been from South America, a view that received little or no support from the mitochondrial evidence (Sykes et al. 1995; Bonatto et al. 1996).

Although the evidence is therefore strong that Polynesians derive most of their maternal lineages from Southeast Asia, a fourth hypothesis has received little attention. This view, in contrast to the “express train” model of an agricultural expansion from Taiwan, suggests that the Austronesian speakers originated neither in southern China nor in Taiwan but toward the center of island Southeast Asia, in the vicinity of the Sulawesi-Mindanao region of the Philippines and Indonesia (Solheim 1994) or perhaps over the entire region of island Southeast Asia in which Austronesian languages are now spoken (Meacham 1984–85). This would suggest that the extant inhabitants of island Southeast Asia were the descendants of earlier Pleistocene settlers rather than of Neolithic people from the mainland. Meacham (1984–85) cites the paucity of extant Austronesian speakers on the southern Chinese mainland—or, indeed, any historical evidence for their existence there—in support of this view. There is also anthropometric evidence that Polynesians closely resemble island Southeast Asian populations but not aboriginal Taiwanese or southern Chinese populations (Pietrusewsky 1997).

Combining the published mitochondrial evidence al-