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COMPREHENSIVE MOLECULAR CYTOGENETIC ANALYSIS OF BARBARY MACAQUE (*MACACA SYLVANUS*)

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Origin of human and ape chromosomes has been studied by comparative chromosome banding analysis and by fluorescence in situ hybridization (FISH). As it is not always possible to determine exact breakpoints and distribution or orientation of specific DNA stretches by these approaches FISH-banding was applied in the present study to reanalyze the chromosomes of Barbary macaque (*Macaca sylvanus*). Interestingly, the results agree with those of previous studies in other macaques, supporting the idea that the genetic differences leading to the observed large morphological differences within the Ceropithecoidae still have to be discovered.

Barbary macaque – FISH – multicolor banding (MCB) – chromosomal breakpoints

Մարդու և կապիկների քրոմոսոմների ծագումը ուսումնասիրվել է քրոմոսոմների համեմատական դիֆերենցիալ գունավորման և ֆլյուորեսցենտային in situ հիբրիդացման (FISH) միջոցով:

Քանի որ նշված մոտեցումները միշտ չէ, որ թույլ են տալիս հստակ որոշել կտրվածքների կետերը և ԴՆԹ-ի սպեցիֆիկ հատվածների բաշխումն ու տեղակայումը, տվյալ հետազոտության մեջ Մագրիբյան մակակ (*Macaca sylvanus*) քրոմոսոմների կրկնակի վերլուծության համար կիրառվել է դիֆերենցիալ FISH-ներկում: Հետաքրքրական է, որ արդյունքները համընկնում են այլ մակակների նախորդ հետազոտությունների արդյունքներին՝ հաստատելով Ceropithecoidae ներսում խոշոր ձևաբանական փոփոխություններին հանգեցնող գենետիկական տարբերությունների հետագա ուսումնասիրության անհրաժեշտությունը:

Մագրիբյան մակակ – FISH – քրոմոսոմների բազմագույն շերտավոր ներկում – քրոմոսոմների կտրվածքներ

Было изучено происхождение хромосом человека и обезьяны с применением сравнительной дифференциальной окраски хромосом и флюоресцентной in situ гибридизации (FISH).Так как указанные подходы не всегда позволяют точно определять точки разрывов и

распределение или ориентацию специфических участков ДНК, в данном исследовании для повторного анализа хромосом магрибского макака (*Macaca sylvanus*) была применена дифференциальная FISH-окраска. Интересно, что полученные данные согласуются с результатами предыдущих исследований других макак и подтвеждают необходимость дальнейших исследований генетических различий, обуславливающих крупные морфологические различия, внутри Ceropithecoidae.

Магрибский макак – FISH – Многоцветная дифференциальная окраска хромосом – хромосомные разрывы

Cytogenetic studies in ape species were done to great extent in the 1970s to 1980s [18] as "comparative cytogenetic studies of non-human primates can provide a substantial contribution to investigations on the evolutionary history of chromosomes and a better understanding of primate and human phylogeny" [9]. Later on the invent of molecular cytogenetics, especially multicolor-fluorescence in situ hybridization (FISH) studies using whole chromosome painting probes gave another boost for studies on chromosomal changes during primate evolution (for review see [10]). Still the FISH-banding approaches available since end of the 1990s [7] were yet applied neither in many species (see below), nor in systematic studies for the question of karyotype evolution, with a few exceptions [5; 12-13]. The nowadays most frequently applied FISH-banding approach is the so-called multicolor banding (MCB) approach, which is anchored in the human DNA-sequence [17]. MCB was already successfully applied for the characterization and comparative molecular cytogenetic mapping of the following primate species before: Gorilla gorilla [10], Hylobates lar [11] and Trachypithecus cristatus [6].

Here we provide the first MCB-based study for the characterization of the North-African Barbary macaque (Macaca sylvanus = MSY). Macaques belong to the Old World monkeys (Catarrhini), family Ceropithecoidae, subfamily Cercopithecinae and tribe Papionini. It is thought that the genus Macaca underwent a radiation in Pliocene or Pleistocene, i.e. during the last 3-5 million years [3]. While morphologically the genus Macaca underwent multiple changes, on the chromosomal level this group kept surprisingly constant – all of them have 42 chromosomes and on cytogenetic level they do not differ at all [2]. This fact is also supported that different macaque species can form hybrids, even fertile ones, easily [8].

The MSY was studied yet only by banding cytogenetics and FISH using whole chromosome paints [9]. As even in times of next generation sequencing basic cytogenetic data is needed for exact alignment of the new complex datasets [19] here we provide the first MCB-based FISH-banding study in MSY. The hereby obtained data is crucial for further comparative cytogenetic studies in non-human primates and their evolutionary history.

Materials and methods. Peripheral blood of MSY (1 male and 1 female) was acquired in the zoological gardens of Erfurt and Hodenhagen (both Germany), respectively. The corresponding veterinaries acquired blood for the present study only if blood collection was necessary anyway for other medical reasons during routine checkups of the animals. Blood lymphocytes from heparinzed blood were subjected to short term culture and cytogenetic work-up using standard procedures.

24 chromosome-specific MCB probes were applied in 24 independent FISH-experiments in MSY-chromosome-preparations as previously reported [10]. Evolutionary conserved chromosomal breakpoints were characterized with respect to the human chromosome complement (see tab. 1). Nomenclature of MSY chromosomes was adapted from [9].

Results and Discussion. Applying MCB in MSY overall 43 evolutionary break-events in comparison to human karyotype were recorded (tab. 1). The observed break-

points were observed to be identical to those known from other macaque species before [16]. Still it has to be admitted that the nomenclature of macaque chromosomes is not uniform – e.g. MSY chromosomes 12 and 13 have the designations 9 and 15 in [15], which may confuse.

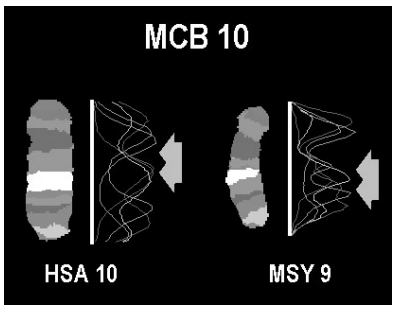

Fig. 1. Representative example for MCB-result obtained in MSY. Result of MCB 10 probe set applied on a human chromosome 10 (HSA) compared to the result obtained on homologous MSY chromosome 9. A paracentric inversion with evolutionary conserved breakpoints in 10q11.23 and 10q22.3 was observed (arrows). For each chromosome pseudocolor depiction and underlying fluorochrome profiles are shown.

Tab. 1. Breakpoints of Macaca sylvanus (MSY) according to MCB. Abbreviations: cen = centromeric position; HSA = Homo sapiens

MSY	MSY chromosomes given as derivatives of human chromosomes	cen
1	inv(1)(q23.3q42.13),dim(1)(q12)	1q42.13
2	der(3)(qter->q27.3::p22.3->p24::q22.1->q27.3::p22.3->p12.3::p26.3->p24::q22.1->p12.3:)	3q26.1
3	der(7)(21qter->21q11.2::7p22.3->7p22.1::7q21.3->7q22.1::7q11.23->7p21.3::7p21.3->7q11.23::7q22.1->7qter)	like HSA 7
4	inv(6)(p24q25.2) and inv(6)(q21q25.2)	6q24.3
5	inv(4)(p15.3q10)	like HSA 4
6	no change to HSA 5	like HSA 5
7	der(15)t(14;15)(q11.2;q26.3)	15q25
8	no change to HSA 8	like HSA 8
9	inv(10)(q11.23q22.3)	like HSA 10
10	der(20)(22qter->22p13::20p11.21->20p13::20q11.21->20qter)	like HSA 22
11	no change to HSA 12	like HSA 12
12	inv(2)(q14.1q21.1)	2q22.1
13	inv(2)(q11.1q14.1)	2p11.2
14	inv(11)(p15.4q13.4)	11p15.4
15	der(9)(9qter->9q34::?::9q34->9p24.3::9q21.11->9q22.33:),dim(9)(q12)	9q33.2
16	der(17)(pter->p10::?::p10->q12::q23.3->q21.32::q12->q21.32::q23.3->q24::?::q24->qter)	like HSA 17
17	no change to HSA 13	13q21.31
18	no change to HSA 18	18q21.2
19	no change to HSA 19	like HSA 19
20	inv(16)(q22.1q22.3),dim(16)(q11.2)	like HSA 16
X	no change to HSA X	like HSA X
Y	del(Y)(q12q12)	like HSA Y

Apart from that, species specific amplifications present in human in 1q12, 9q12, 16q11.2 and Yq12 were not present in MSY at the corresponding regions. Still unknown material was amplified in MSY in regions homologous to 17p10, 17q24 and 9q34. At least for 17q24 complex regions of segmental duplication were reported [4]. Such species specific amplifications are suggested to play major roles in speciation [10].

Centromeric regions present in MSY were identical to human centromeric positions in MSY 3, 5, 6, 8-11, 16, 19, 20, X and Y (tab. 1). It is well known that the centromeric regions, even if being intraspecifically stable do not contain identical alphoid DNA stretches [1]; this is thought to be a hint on faster evolution of these genomic regions compared to other, euchromatic ones.

Different centromeric positions than in human were observed in the MSY chromosomes 1, 2, 4, 7, 12-15, 17 and 18 (tab. 1). The latter was denominated a centromere repositioning and was thoroughly discussed in [14].

Overall, the present study confirmed for another macaque species that the general chromosomal composition cannot be the reason for specification in this genus.

The present study in MSY using high resolution FISH-banding underlined the conclusion already drawn in [9] that "the karyotype has not played a fundamental role in the diversification and speciation in this group, because apparently there is no necessary causal link between chromosomal changes and morphological diversification or speciation". In other words: the genetic differences leading to large morphological differences within the Ceropithecoidae still have to be identified. At the same time it needs to be revised if the family Hylobatidae really is as morphological inconsistent due to its extremely chromosomal diversity [11] or due to other, subchromosomal changes.

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REFERENCES

1. *Archidiacono N., Antonacci R., Marzella R., Finelli P., Lonoce A., Rocchi M.* Comparative mapping of human alphoid sequences in great apes using fluorescence in situ hybridization. Genomics, *25*, 477-484, 1995.
2. *Brown C.J., Dunbar V.G., Shafer D.A.* A comparison of the karyotypes of six species of the genus Macaca and a species of the genus Cercocebus. Folia Primatol (Basel), *46*, 164-172, 1986.
3. *Camperio Ciani A., Stanyon R., Scheffrahn W., Sampurno B.* Evidence of gene flow between Sulawesi macaques. Am J Primatol, *17*, 257-270, 1989.
4. *Cardone M.F., Jiang Z., D'Addabbo P., Archidiacono N., Rocchi M., Eichler E.E., Ventura M.* Hominoid chromosomal rearrangements on 17q map to complex regions of segmental duplication. Genome Biol, *9*, R28, 2008.
5. *de Oliveira E.H., Neusser M., Figueiredo WB, Nagamachi C., Pieczarka J.C., Sbalqueiro I.J., Wienberg J., Müller S.* The phylogeny of howler monkeys (Alouatta, Platyrrhini): reconstruction by multicolor cross-species chromosome painting. Chromosome Res, *10*, 669-683, 2002.
6. *Fan X., Pinthong K., Mkrtchyan H., Siripiyasing P., Kosyakova N., Supiwong W, Tanomtong A., Chaveerach A., Liehr T., de Bello Cioffi M, Weise A.* First detailed reconstruction of the karyotype of Trachypithecus cristatus. Mol Cytogenet, *in press.*
7. *Liehr T., Starke H., Heller A., Kosyakova N., Mrasek K., Gross M., Karst C., Steinhaeuser U., Hunstig F., Fickelscher I., Kuechler A., Trifonov V., Romanenko S.A., Weise A.* Multicolor fluorescence in situ hybridization (FISH) applied to FISH-banding. Cytogenet Genome Res, 114, 240-244, 2006.
8. *Moore C.M., Janish C., Eddy .CA., Hubbard G.B., Leland M.M., Rogers J.* Cytogenetic and fertility studies of a rheboon, rhesus macaque (Macaca mulatta) x baboon (Papio hamadryas) cross: further support for a single karyotype nomenclature. Am. J. Phys. Anthropol, *110*, 119-127, 1999.

9. *Morescalchi A.M., Camperio Ciani A., Stanyon R.* Chromosome banding and molecular cytogenetics of the Barbary macaque, Macaca sylvanus. It J Zool, *65*, 101-107, 1998.
10. *Mrasek K., Heller A., Rubtsov N., Trifonov V., Starke H., Rocchi M., Claussen U., Liehr T.* Reconstruction of the female Gorilla gorilla karyotype using 25-color FISH and multicolor banding (MCB). Cytogenet Cell Genet, *93*, 242-248, 2001.
11. *Mrasek K., Heller A., Rubtsov N., Trifonov V., Starke H., Claussen U., Liehr T.* Detailed Hylobates lar karyotype defined by 25-color FISH and multicolor banding. Int. J. Mol. Med., *12*, 139-146, 2003.
12. *Müller S., Wienberg J.* "Bar-coding" primate chromosomes: molecular cytogenetic screening for the ancestral hominoid karyotype. Hum Genet, *109*, 85-94, 2001.
13. *Müller S., O'Brien P.C., Ferguson-Smith M.A., Wienberg J.* Cross-species colour segmenting: a novel tool in human karyotype analysis. Cytometry, *33*, 445-452, 1998.
14. *Rocchi M., Archidiacono N., Schempp W., Capozzi O., Stanyon R.* Centromere repositioning in mammals. Heredity (Edinb), *108*, 59-67, 2012.
15. *Ruiz-Herrera A., Ponsà M., García F., Egozcue J., García M.* Fragile sites in human and Macaca fascicularis chromosomes are breakpoints in chromosome evolution. Chromosome Res., *10*, 33-44, 2002.
16. *Ventura M., Antonacci F., Cardone M.F., Stanyon R., D'Addabbo P., Cellamare A., Sprague L.J., Eichler E.E., Archidiacono N., Rocchi M.* Evolutionary formation of new centromeres in macaque. Science, *316*, 243-246, 2007.
17. *Weise A., Mrasek K., Fickelscher I., Claussen U., Cheung S.W., Cai W.W., Liehr T., Kosyakova N.* Molecular definition of high-resolution multicolor banding probes: first within the human DNA sequence anchored FISH banding probe set. J. Histochem. Cytochem., *56,* 487-493, 2008.
18. *Yunis J.J., Prakash O.* The origin of man: a chromosomal pictorial legacy. Science, *215*, 1525-1530, 1982.
19. *Zhang X., Goodsell J., Norgren R.B. Jr.* Limitations of the rhesus macaque draft genome assembly and annotation. BMC Genomics, *13*, 206, 2012.

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