Heliyon

ELSEVIER

Received:
15 September 2015
Revised:
20 October 2015
Accepted:
23 October 2015

Heliyon (2015) e00042

Comprehensive characterization of evolutionary conserved breakpoints in four New World Monkey karyotypes compared to *Chlorocebus aethiops* and *Homo sapiens*

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Abstract

Comparative cytogenetic analysis in New World Monkeys (NWMs) using human multicolor banding (MCB) probe sets were not previously done. Here we report on an MCB based FISH-banding study complemented with selected locus-specific

http://dx.doi.org/10.1016/j.heliyon.2015.e00042

and heterochromatin specific probes in four NWMs and one Old World Monkey (OWM) species, i.e. in *Alouatta caraya* (ACA), *Callithrix jacchus* (CJA), *Cebus apella* (CAP), *Saimiri sciureus* (SSC), and *Chlorocebus aethiops* (CAE), respectively. 107 individual evolutionary conserved breakpoints (ECBs) among those species were identified and compared with those of other species in previous reports. Especially for chromosomal regions being syntenic to human chromosomes 6, 8, 9, 10, 11, 12 and 16 previously cryptic rearrangements could be observed. 50.4% (54/107) NWM-ECBs were colocalized with those of OWMs, 62.6% (62/99) NWM-ECBs were related with those of *Hylobates lar* (HLA) and 66.3% (71/107) NWM-ECBs corresponded with those known from other mammalians. Furthermore, human fragile sites were aligned with the ECBs found in the five studied species and interestingly 66.3% ECBs colocalized with those fragile sites (FS). Overall, this study presents detailed chromosomal maps of one OWM and four NWM species. This data will be helpful to further investigation on chromosome evolution in NWM and hominoids in general and is prerequisite for correct interpretation of future sequencing based genomic studies in those species.

Keywords: Genetics, Evolutionary genetics, Evolutionary conserved breakpoints, Multicolor banding, New World Monkeys, Old World Monkeys, Fragile sites, Atelidae, Cebidae

Abbreviations: ACA: *Alouatta caraya*, BACs: bacterial artificial chromosomes, CAE: *Chlorocebus aethiops*, CJA: *Callithrix jacchu*s, CAP: *Cebus apella*, EC: evolutionary conserved, ECBs: evolutionary conserved breakpoints, FISH: fluorescence in situ hybridization, FS: fragile site, HCM: heterochromatin mix, HLA: *Hylobates lar*, HSA: *Homo sapiens*, HSBs: homologous syntenic blocks, MCB: multicolor banding, NGS: Next-generation sequencing, NOR: nucleolus organizer region, NWMs: New World Monkeys, OWMs: Old World Monkeys, SSC: *Saimiri sciureus*, subCTM: sub-centromere/subtelomere-specific multicolor (FISH), wcp: whole human chromosome painting

1. Introduction

New World Monkeys (NWMs) inhabit tropical forests of Southern Mexico, central and South America, but especially the Amazon rainforests. Nowadays, there are over 120 recognized species that comprise of over 16 genera, commonly classified in 3 families. It is known from cytogenetic studies that NWMs have high interchromosomal and intrachromosomal karyotypic diversity in terms of chromosome structure and numbers, the latter ranging from $2n = 16$ to $2n = 62$ (Groves, 2001).

Four NWM species from two families are included in this study: *Alouatta caraya* (ACA) from the family Atelidae, and *Callithrix jacchus* (CJA), *Cebus apella* (CAP), *Saimiri sciureus* (SSC) from the family Cebidae. These species

http://dx.doi.org/10.1016/j.heliyon.2015.e00042

have been previously investigated for their chromosomal organization, however primarily just by application of cytogenetic techniques, such as G-banding analyses in CAP (Freitas and Seuánez 1982), CJA (Ardito et al., 1987), ACA (Rahn et al., 1996) and SSC (Srivastava et al., 1969), C-banding in CAP (Freitas and Seuánez 1982), CJA (Bedard et al., 1978), ACA (Mudry et al., 1994) and SSC (Jones and Ma 1975), and Ag-NOR staining in CAP (Freitas and Seuánez 1982), CJA (Bedard et al., 1978), ACA (Mudry et al., 1994) and SSC (Goodpasture and Bloom, 1975). Since the 1990s, fluorescence in situ hybridization (FISH) applying whole human chromosome painting (wcp) probes and/or monkeys chromosome-specific probes have been successively utilized for comparative cytogenetics studies in NWMs, like CAP (Richard et al., 1996; Garcia et al., 2000), CJA (Sherlock et al., 1996; Neusser et al., 2001), ACA (de Oliveira et al., 2002) and SSC (Stanyon et al., 2000).

Apart from NWMs, Old World Monkeys (OWMs) and apes also were subject of research before using cytogenetics and FISH; examples are chimpanzees, gorillas, orangutans, lesser apes, African green monkey (*Chlorocebus aethiops* = CAE), macaques and langurs (Stanyon et al., 1992; Wienberg et al., 1990, 1992; Luke and Verma, 1992; Ried et al., 1992; Koehler et al., 1995; Bigoni et al., 1997; Finelli et al., 1999). While chromosomal diploid numbers and evolutionary conserved rearrangements could be determined or at least suggested already based on cytogenetics, FISH painting using wcp probes made it possible to investigate a wide range of interchromosomal translocations which took place during hominoid-evolution. However, the relatively limited resolution of wcp probes hampered detection of smaller rearrangements and intrachromosomal changes, like inversions. This could be overcome by FISH-banding approaches (Liehr et al., 2006) like multicolor banding (MCB) (Liehr et al., 2002; Weise et al., 2008). MCB was successfully applied for comparative mapping of the following primate species before: *Gorilla gorilla* (Mrasek et al., 2001), *Hylobates lar* (Mrasek et al., 2003), *Trachypithecus cristatus* (Fan et al., 2013), *Macaca nemestrina* (Fan et al., 2014a), *Macaca sylvanus* (Fan et al., 2014b) and *Macaca fascicularis* (Fan et al., 2014c).

In this study, we determined the chromosomal structure of the following 4 NWM species: CAP (2n = 54), CJA (2n = 46), ACA (2n = 50) and SSC (2n = 44), as well as of one species OWM-species CAE (2n = 60). Besides MCB, bacterial artificial chromosomes (BACs) have been used for identification of centromeric positions and possible cryptic aberrations. Furthermore, positions of evolutionary conserved breakpoints (ECBs) among the studied OWM and NWM species were compared to data from the literature. Finally, a new phylogenetic tree was suggested based on the ECBs and EC rearrangements found in this study.

http://dx.doi.org/10.1016/j.heliyon.2015.e00042

2. Material and methods

2.1. Cell culture and chromosomal preparation

Immortalized lymphoblast cell lines derived from tufted capuchin monkey (*Cebus apella*, CAP; female), common marmoset (*Callithrix jacchus*, CJA; female), howler monkey (*Alouatta caraya*, ACA; female), squirrel monkey (*Saimiri sciureus*, SSC; female) and the African green monkey (*Chlorocebus aethiops*, CAE; female) were cultivated according to standard techniques. Chromosomes were prepared following standard protocols (Mrasek et al., 2001).

2.2. Fluorescence in situ hybridization (FISH)

FISH was done as previously reported using locus-specific bacterial artificial chromosomes (BAC) probes and/or multicolor banding (MCB) probe sets (Fan et al., 2013). Also all chromosome-specific sub-centromere/subtelomere-specific multicolor (subCTM-)FISH probe sets were applied (Gross et al., 2006) apart the Y-chromosome specific one, as only female cell lines were available. Additionally, the following homemade *Homo sapiens* (HSA) derived microdissection probes were utilized: a probe specific for the short arm of all human acrocentric chromosomes (midi54) (Mrasek et al., 2003), and partial chromosome paints for some selected chromosome-arms (Liehr and Claussen, 2002). Furthermore, a probe specific for the nucleolus organizer region (NOR) and a probe set directed against all heterochromatic regions present in the human genome (1q12, 16q11.2, 9q12, 9p12/ 9q13, 15p11.2-p11.1, 19p12/q12 and Yq12), the so-called heterochromatin mix (HCM) probe set (Bucksch et al., 2012) were utilized.

Images were captured by an Axioplan II microscope (Carl Zeiss Jena GmbH, Germany) equipped with filter sets for DAPI, FITC, TR, SO, Cy5 and DEAC. Image analysis was performed via pseudocolor banding and fluorochrome profiles of the ISIS digital FISH imaging system (Meta Systems Hard & Software GmbH, Altlussheim, Germany). A total of 10 up to 20 metaphases per species and probe were analyzed.

3. Results

Multicolor banding (MCB) using human chromosome-specific probe sets was successfully applied in all five here studied species. Results were obtained for all chromosomes excluding Y-chromosome, as only female individuals were available for this study. ECBs and centromeric positions could be estimated at the sub-band level. Fig. 1 and Supplementary Table S1 summarizes the obtained results.

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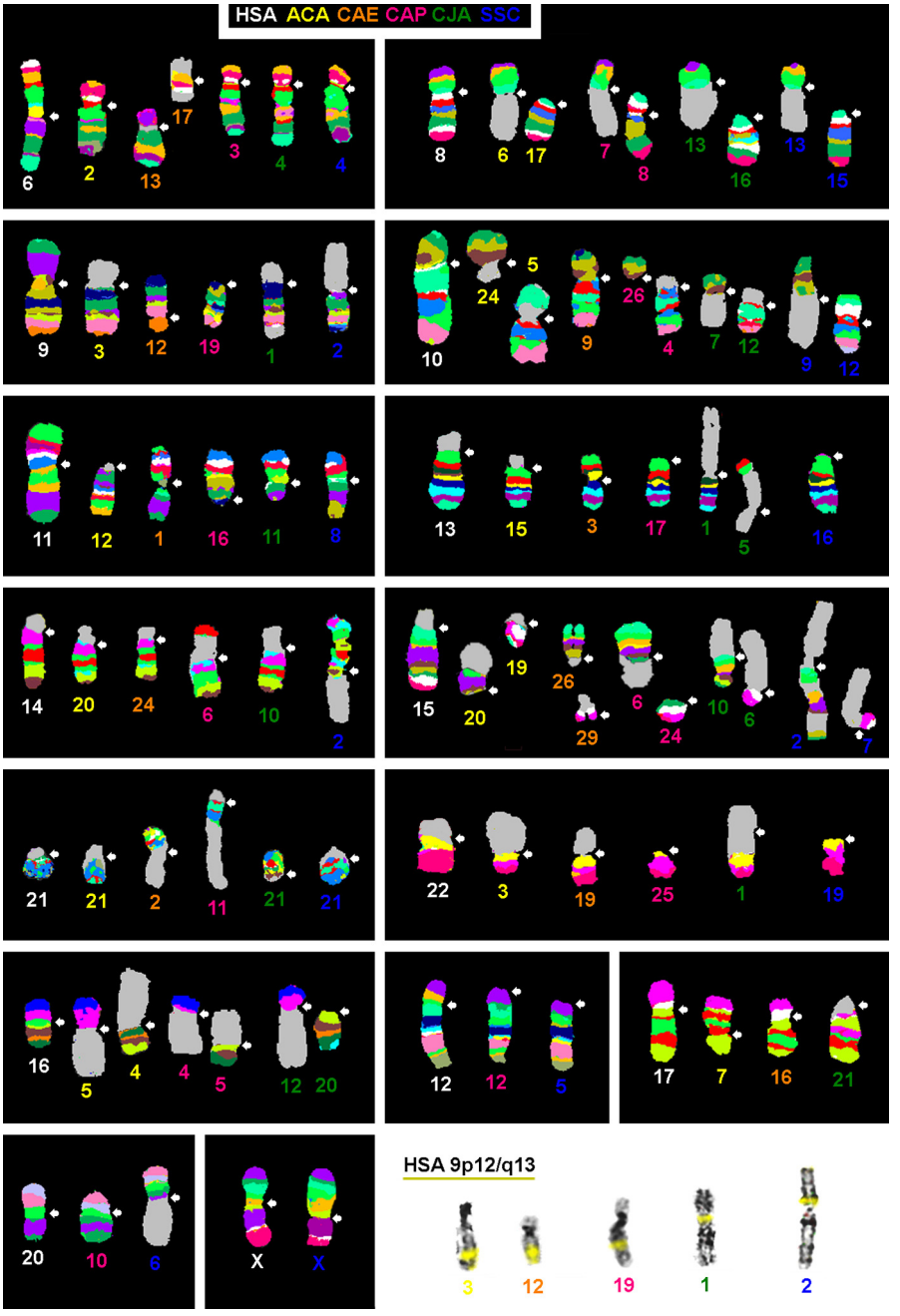

Fig. 1. Representative MCB pseudo-color results using human probes on the five species studied here; depicted are only new, not including confirmatory results of previously published findings from others. HSA chromosomes are numbered by white figures, monkey chromosomes in other colors. Also FISH-results using HSA 9p12/9q13 probes in the five studies species are depicted in the bottom right corner. Arrows show the location of monkey centromere.
Abbreviations: ACA = *Alouatta caraya*, CAE = *Chlorocebus aethiop*s, CAP = *Cebus apella*, CJA = *Callithrix jacchus*, HSA = *Homo sapiens*, SSC = *Saimiri sciureus*.

http://dx.doi.org/10.1016/j.heliyon.2015.e00042

Besides MCB probes specific for all sub-centromeric and sub-telomeric regions in HSA were also applied (results not shown). Some of centromeric positions were flanked by sub-centromeric probes and exactly mapped; remaining centromeric positions could be narrowed down by MCB (Supplementary Table S1). None of the sub-telomeric regions were involved in, compared to human, cryptic rearrangements during evolution of the five studied species (results not shown). Also, apart from 3 exceptions none of the human specific heterochromatic regions covered by the HCM probe-sets could be aligned to the homologous regions in the five studied species: signals for the HSA-specific probe covering 19p12/19q12 were observed on CAP9, ACA8, CJA22, SSC14 and CAE6, respectively; NOR-specific probe and probe specific for HSA 9p12/9q13 corresponded to chromosomes CAP19, ACA3, CJA1, SSC2 and CAE12, respectively (Fig. 1).

In total, in all five studied species, 363 ECBs which cannot be observed in HSA (Supplementary Table S2) and 253 homologous syntenic blocks (HSBs) were identified in this study. Practically all chromosomes studied underwent at least one rearrangement in the studied species compared to human (Supplementary Table S1; Table 1). As substantial parts of the overall observed 363 ECBs were seen in two or all of the studied species overall 107 different ECBs were identified (Supplementary Table S2).

Centromeric regions were either (i) de novo as interstitial ones within evolutionary conserved blocks, (ii) de novo formed in ECBs and/or break/fusion points or (iii) conserved compared to regions homologous to human centromeres. Also (iv) the latter two types could seed two centromeric positions. All four types of centromere positioning were found in all five studied species to different extents (Supplementary Table S1).

An analysis of HSBs based on detected ECBs on the five studied species is shown in Fig. 2. HSB rates were different per homologous HSA-chromosomes and species. E.g. HSA3 had much more HSBs than similar sized HSA1 and HSA2 chromosomes. The overall tendency is that the number of ECBs decreased with the size of the human chromosomes. Overall SSC had the most while CAP had the smallest number of HSBs compared to HSA.

The ECBs of CAP, ACA, CJA, SSC and CAE from Supplementary Table S1 were further compared with ECBs in other species (Supplementary Table S2). 50.4% (54/107) NWM-ECBs were colocalized with those OWM, 62.6% (62/99) NWM-ECBs were related with those of HLA and 66.3% (71/107) NWM-ECBs corresponded with those known from other mammalians, based on Supplementary Table S2. Furthermore, human fragile sites (FS) were aligned with the ECBs found in the five studied species and interestingly 66.3% ECBs colocalized with those FS.

http://dx.doi.org/10.1016/j.heliyon.2015.e00042

Table 1. Evolutionary conserved breakpoints as found in the present study; those used for designing of Fig. 3 are highlighted by asterisk.

Homologues in HSA	CAP		CJA		SSC		ACA		CAE	
	hom.	abbr.	hom.	abbr.	hom.	abbr.	hom.	abbr.	hom.	abbr.
1	14	der(1) 1*	7	t(1;10)	11	der(1) 1*	1	t(1;5)	20	–
1	22	der(1) 2*	19	der(1) 2*	18	der(1) 2*	23	der(1) 2*	20; 25	–
1	23	–	18	–	14	t(1;19)	22	–	20	–
3	20	–	17	–	6	20 inv t(3;20)*	X2	t(3;15)*	15	–
3; 21	11	t(3;21)*	21	t(3;21) cen*	21	t(3;21)*	21	t(3;21)*	2; 22	–
4	2	–	3	–	3	4 inv	4; 9; 19	4 compl	7; 27	4 fi
7	15	–	8	7 inv1	10	7 inv2	14	–	21	–
8	8	del(8)*	16	del(8) inv*	15	del(8)*	17	del(8)*	8	–
8; 18	7	t(8;18)*	13	t(8;18)*	13	t(8;18)*	6	t(8;18)*	8; 18	–
10	26	–	7	t(1;10)	9	t(3;10)	24	–	9	–
10; 16	4	t(10;16)*	12	t(10;16)*	9; 12	t(10;16)*	5	t(10;16) inv*	5; 9	10 compl
12	12	12 inv*	9	–	5	12 inv	11	–	11	–
13	17	–	5	t(13;17)*	16	–	15	–	3	–
14; 15	6	t(14;15)* inv	10	t(14;15)*	2	t(14;15) compl*	19; 20	t(14;15) fi*	24; 26	–
16	5	t(2;16)*	20	–	1	t(2;16;5)*	4; 16	t(4;16)	5	–
17	21	17 inv2	5	t(17;20)	17	–	7	–	16	17 inv1
19	9	–	22	–	14	t(1;19)	8	–	6	–
20	10	20 inv*	5	t(17;20)	6	20 inv t(3;20)*	10	t(2;20)	2	t(2;21)
22	25	–	1	t(9;22)*	19	–	3	t(9;22)*	19	–
X	X	–	X	–	X	X neo	X1	–	X	–

Abbreviations: abbr. = abbreviation as used in Fig. 3; hom. = homologous chromosome(s); ACA = *Alouatta caraya*; CAE = *Chlorocebus aethiops*; CAP = *Cebus apella*; CJA = *Callithrix jacchus*; SSC = *Saimiri sciureus*; HSA = *Homo sapiens*; NWM = New World Monkey; OWM = Old World Monkey; t = translocation, del = deletion, der = derivative chromosome, inv = inversions, fi = fission; neo = neo-centromere.

For Capuchin monkey (*Cebus apella*, CAP) 43 ECBs were identified by FISH-banding (Supplementary Table S1). Additionally there are eight conserved centromeric regions compared with human (i.e. HSA1, HSA13, HSA16, HSA19, HSA20, HSA22, HSAX). Twenty-three regions were identified as de novo centromeres: twenty presented at break/fusion points (details see the Supplementary Table S1), three in the middle of conserved chromosomal block (i.e. 4q32.1, 5q31.3, 8q21.13). The regions homologous to 15q24.1 as well as centromeric region homologous to HSA1 were used for seeding of two centromeric positions, each (Supplementary Table S1).

Howler monkey (*Alouatta caraya*, ACA) had 51 ECBs and interestingly, ACAX2 chromosome was delineated as der(3)

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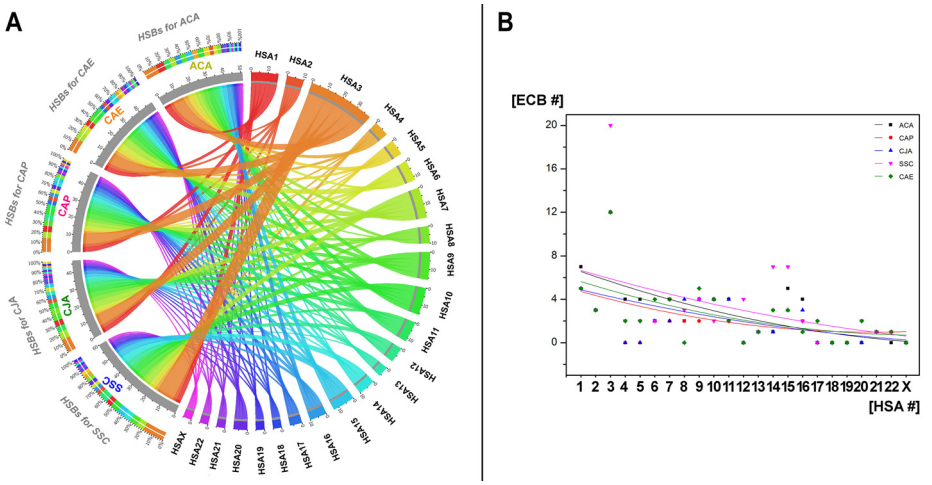

Fig. 2. Analysis of homologous syntenic block and evolutionary conserved breakpoints (ECBs) on the five studied species. A: The linkage map shows homologous syntenic blocks (HSB) of HSA chromosomes 1-22 and X compared to ACA = *Alouatta caraya*, CAE = *Chlorocebus aethiops*, CAP = *Cebus apella*, CJA = *Callithrix jacchus*, HSA = *Homo sapiens*, SSC = *Saimiri sciureus*. HSB rates per chromosome and species are shown. B: The graph shows the distribution of breakpoints in five studied monkeys with respect to the human chromosomes (colored dots), and the calculated breakpoints tendency curve (lines). As expected the number of breakpoints decreased with the size of the human chromosomes. SSC had in this study compared to HSA the most ECBs, CAP the smallest number of ECBs.

(15qter→15q24.3::3q27.1→3q22.1::3p25.3→3p21.31:). Five centromeric regions remained conserved compared to human (HSA1, HSA8, HSA13, HSA19, HSAX). Twenty-seven regions were identified as de novo centromeres: twenty-three formed at break/fusion points (details see the Supplementary Table S1), four were again in the middle of conserved blocks (i.e. 4q34.1, 10p11.21, 12p13.3 and 17q23.2); the regions homologous to 2q14.3 and 15q24.1 seeded two centromeric positions (Supplementary Table S1).

Common Marmoset (*Callithrix jacchus*, CJA) had 48 ECBs in a chromosome set of 46. Also there are ten centromeric regions conserved compared to human (HSA1, HSA3, HSA9, HSA12, HSA14, HSA15, HSA16, HSA19 and HSAX). Neocentromeres formed in twenty regions; seventeen are present at break/fusion points, three are interstitial in conserved blocks (i.e. 4q32.1, 5q31.3 and 7p21.1), and two regions (2q14.3 and HSA 16) seeded again two centromeric positions, each (Supplementary Table S1).

FISH-results for Squirrel monkey (*Saimiri sciureus*, SSC) are summarized in Supplementary Table S1: there are 67 ECBs in this species compared to HSA. Six centromeres were conserved compared to human (HSA1, HSA13, HSA15, HSA16 and HSA22). Twenty-three regions were identified as de novo centromeres of which nineteen are located at break/fusion points, the remainder

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centromeres are interstitial in conserved blocks (i.e. 4q32.1, 10q26.3, 11q12.1 and Xq25). Finally, HSA 1 was used for seeding of two centromeric positions (Supplementary Table S1).

Results for African green monkey (*Chlorocebus aethiops*, CAE) and its 60 CAE chromosomes 39 ECBs can be found in Supplementary Table S1. In CAE there are eleven conserved centromeric regions compared to human (HSA2, HSA5, HSA8, HSA10, HSA12, HSA14, HSA16, HSA17, HSA19, HSA20, HSAX). De novo centromeres formed in 21 regions: fourteen are present at break/fusion points, there are seven interstitial ones in conserved blocks (i.e. 2q14.3, 3q26.33, 4q13.1, 6p11.2, 9q34.13, 13q21.31, 18q21.1), and two regions (7q11.21 and 15q24.1) seeded two centromeric positions, each (Supplementary Table S1).

4. Discussion

This study comprehensively characterized by high resolution molecular cytogenetics four species from NWMs derived from family Atelidae and Cebidae. Also one OWM species from the Cercopithecini was selected for the present study. This combination of species was done considering the assumption that NWM ancestors came from Africa. This idea was based on basis of morphological resemblance between NWM and the African anthropoid fossils (Schrago and Russo, 2003) and the African rafting source theory (Kay 2015). However, the present study did not find gross similarities between CAE from OWMs and the studied NMW species. Thus, CAE may have common ancestors with the studied NWMs but is no relative with many EC rearrangements in

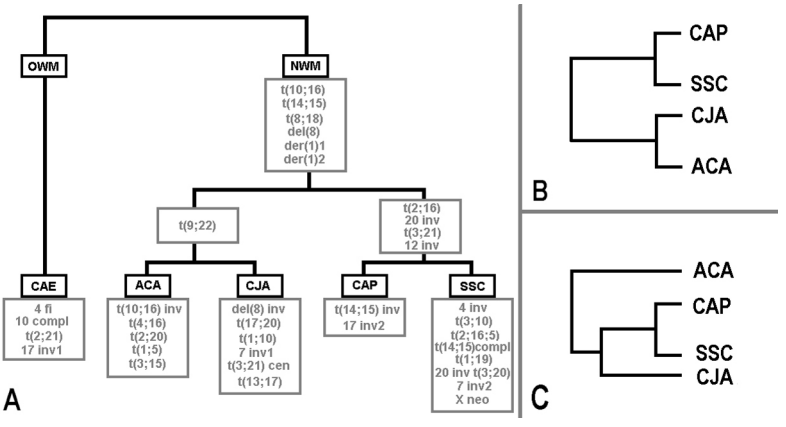

Fig. 3. A) Based on the here described evolutionary conserved changes a putative pedigree for the 4 NMM and one OWM is provided. B) The same putative pedigree according to Ford (1986) and C) Perelman et al. (2011) suggesting the same as Finstermeier et al. (2013).
Abbreviations: ACA = *Alouatta caraya*; CAE = *Chlorocebus aethiops*; CAP = *Cebus apella*; CJA = *Callithrix jacchus*; SSC = *Saimiri sciureus*; NWM = New World Monkey; OWM = Old World Monkey; t= translocation, del = deletion, der= derivative chromosome, inv= inversions, fi= fission; neo= neo-centromere.

http://dx.doi.org/10.1016/j.heliyon.2015.e00042

common (Table 1 and Fig. 3) Also available previously published own data on karyotypes of Macaques (tribe Papionini) (Fan et al., 2014a,b,c) *Trachypithecus cristatus* (TCR) (Fan et al., 2013), and *Hylobates lar* (HLA) (Mrasek et al., 2003) revealed by identical approaches as used here were included in the present study (Supplementary Table S2).

In general, our results confirmed previous data on homologous regions of the here studied five species and HSA (Garcia et al., 2000; Stanyon et al., 2000; Neusser et al., 2001; de Oliveira et al., 2002), but also we found that homologous regions for HSA chromosomes 6, 8, 10, 11, 12, 16 and 17 underwent at least one rearrangement, each, compared to ACA, CJA, CAP, SSC and CAE, respectively. In fact, 107 evolutionary conserved breakpoints (ECBs) and 253 homologous syntenic blocks (HSB) were determined. Besides multicolor banding (MCB), specific human sub-centromeric and sub-telomeric probes were also applied to identify the distribution of centromeres and telomeres in five species. Some of chromosome centromeric positions were neo-centromeres that were not characterized in previous studies (Garcia et al., 2000; Neusser et al., 2001). Remaining centromeres kept their positions during evolution from common ancestors to HSA. In contrast to previous report in HLA (Weise et al., 2015), none cryptic rearrangements were detected in the sub-telomeric regions during evolution of the five studied species.

Compared to reciprocal chromosome painting or multicolor chromosome bar coding in previous NWM studies (Neusser et al., 2001; Finelli et al., 1999; Müller and Wienberg 2001), MCB technique can be applied for the detailed identification of balanced and unbalanced chromosome rearrangements, even detected intrachromosomal rearrangements, to provide a genome-wide overview of sub-regional organization of syntenic segments and position of breakpoints, changes which are difficult if not impossible to be visualized by chromosome banding (Wienberg 2005). However, mostly sub-telomeric rearrangements and the high plasticity of sub-telomeric regions, in contrast to BAC mapping in HLA study (Misceo et al., 2008) escape the detection by MCB. To overcome this problem, sub-centromere/sub-telomere as locus specific probes also were utilized to check for cryptic rearrangements during evolution of the five studied species. Array-comparative genomic hybridization can only detect precisely map unbalanced rearrangements. Thus it can only be applied in evolutionary studies when combining with glass needle based microdissection like previously shown by us (Weise et al., 2015). Even though possible it is a very laborious approach which was not chosen for the present study.

Next-generation sequencing (NGS) technology was applied in some evolutionary studies (Li et al., 2010; Carbone et al., 2014), however, it is difficult to correctly align sequence and assemble genomes which are extremely reshuffled; thus

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karyotypic data is essentially necessary to correctly understand the NGS-data. Also NGS is not able to annotate information on position of repetitive elements. Therefore the human heterochromatin oriented heterochromatin mix (HCM) FISH set was applied in this study and localized some homologous regions like NOR or regions homologous to 9p12/9q13. Despite monkey specific repetitive elements were failed to be detected, they may be also of importance of evolution. Here also microdissection can be applied, even though this was not used in the present study (own unpublished data).

Overall, MCB combined with sub-centromeric/sub-telomeric probes and HCM-FISH set effectively detected detailed ECBs and orientation of newly arranged chromosomal regions in NWM. In present study, HSA 12, 18, 19, 20 and X were found as most conserved syntenic blocks during evolution. Chromosomes ACA11 and CJA9 were completely homologous to HSA 12. This finding is different from previous reports in other NWM which demonstrated a pericentric inversion (e.g. in *Lagothrix lagotricha*,*Callicebus moloch*, *Saimiri boliviensis*) (Stanyon et al., 2008). In concordance with the literature HSA18 is well conserved throughout mammals (Stanyon et al., 2008), here it was homologous to ACA6, CAP7, CJA13 and SSC13, respectively. Also our data in all five species HSA19 is conserved as syntenic block supports the hypothesis that this block is highly conserved after fusing of 19p and 19q in the anthropoid ancestor including NWM, OWM apes and human (Stanyon et al., 2008). Chromosomes homologous to HSA20 have structural changes due to neocentromere formation, translocations or inversions in ACA10, CAE2, CJA5 and other species (Stanyon et al., 2008). Additionally, we confirmed previous reports that X-chromosome has a centromeric shift in SSC (Rocchi et al., 2012). This finding supported that there are only a few exceptions from an X-chromosome being stable in most NWMs (Stanyon et al., 2008).

Centromere repositioning is a widespread phenomenon in genome evolution and a clustering of segmental duplications around the centromere is a common feature of primate sub-centromeric regions (Eder et al., 2003; Ventura et al., 2007). In this study, sub-telomeric and sub-centromeric probes were selected, which located very close to the telomere or centromere, respectively. Totally, of the centromeres in the studied five species (ACA, CAE, CAP, CJA and SSC), 43% of the centromeres were conserved and mapped between human sub-centromeric probes that flanked the centromeres. It is well known that the centromeric regions do not contain identical alphoid DNA stretches; this is understood as a hint on faster evolution of these genomic regions compared to others, euchromatic ones (Archidiacono et al., 1995). Besides, neocentromeres distinct from HSA centromere position were identified (see details in Supplementary Table S1). Noteworthy, other authors suggested that blocks of segmental duplication locate in close proximity to centromeric satellite DNA;

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these neocentromeres could be thus rapidly stabilized by acquiring alpha satellite DNA (Ventura et al., 2007; She et al., 2004). As previously discussed for HLA (Mrasek et al., 2003) identical regions can be used twice for centromere-seeding and regions being telomeric in HSA can be centromeric positions in other species (Supplementary Table S1). Also Ventura et al. 2004 reported that the centromere of human chromosome 15 occurred in the telomeric region of the short arm of the ancestral chromosome 15/14 association. Possible explanations are that duplicon exchanges between sub-centromeric and sub-telomeric duplications are relatively frequent (Bailey et al., 2002) and that evolutionary new centromere appearance in telomeric regions may be affected by the spread of sub-centromeric duplications (Ventura et al., 2004).

For numbers of identified ECBs and HSBs, as expected the number of both decreased with the size of the human chromosome compared to as a reference (Fig. 2B). In this study CAP had the smallest number of ECBs, compared to HSA. This finding is in concordance with previous reports that the subfamily Cebidae among NWM occupies a more basal position and CAP has conserved chromosomal composition in the ancestral NWM karyotype (Amaral et al., 2008).

50.4% NWM ECBs colocalized with those of OWM and 62.6% NWM ECBs related with those of HLA (Supplementary Table S2). Our data show a higher percentage of ECBs colocalization between NWM and HLA, even though they are distantly related species. One possible explanation is that HLA experienced a high degree of chromosomal rearrangements by rapid derived karyotype evolution, although human and HLA are closely linked by a common ancestor (Weise et al., 2015). Furthermore, 66.3% (71/107) NWM ECBs were identified to correspond with those of mammalians in general (Supplementary Table S2). This finding is consistent with previous reports that 64% human chromosomal bands that contain evolutionary breakpoints presented in seven mammalian species (Ruiz-Herrera et al., 2006). Thus, there must be some 'breakpoint prone regions' in the mammalian genomes, which may be used by evolution as well as in human diseases (Liehr et al., 2011). These regions seem to correlate by large means especially with human FS (Supplementary Table S2) (Mrasek et al., 2010). Our data showing 66.3% of the here detected ECBs colocalized with FS confirmed previous findings that ECB regions are highly relevant to common FS in the breakage frequency model and that expressed FS have a tendency to concentrate at ECBs (Ruiz-Herrera et al., 2006; Fungtammasan et al., 2012).

In the present study, the karyotype of human was compared with chromosomes of CAP, ACA, SSC and CJA. CAE is an OWM considered to have common ancestors with NWMs studied and was used as an outgroup here. The data presented here enabled to follow up the chromosomal evolution among the

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NWMs. Shared chromosomal rearrangements were considered as cladistics markers for linkage. Fig. 3 summarizes a putative pedigree for NWMs analyzed (see also Table 1). The translocation of human 9/22 homologs was observed in both ACA and CJA as a landmark distinct from a sister group of CAP and SSC. And unique translocations of 10/16, 4/16, 2/20, 3/15 and 1/5 in ACA on the sub-chromosomal region level confirmed previous publications (de Oliveira et al., 2012). Furthermore, less number of chromosomal rearrangements was observed in CAP, which proved that CAP at a basal position in NWM depicted before (Neusser et al., 2001; Amaral et al., 2008). Meanwhile, CAP sharing chromosome rearrangements with SSC including two translocations HSA2/16, 3/21 and inversions in HSA20 and HSA12, forming a clade, indicated they have a closer relationship than other two species. This finding is in agreement with previous molecular phylogenetic tree (Finstermeier et al., 2013). The results obtained here are in concordance with previous morphological studies (Ford 1986), however do not fit to recent molecular phylogenetic ones (Perelman et al., 2011; Finstermeier et al., 2013). It is a possible explanation that due to uncoupled molecular and morphological evolution, the likelihood of reconstructing similar phylogenetic relationships was affected. Therefore phylogenetic history merely relied on previous molecular trees need be reevaluate (Perez and Rosenberger, 2014). The controversy of NWM phylogenetic relationship still remains as distinct molecular and morphological datasets, further comparative cytogenetic studies could provide new insights to reach a final conclusion relied on the high resolution of genetic datasets of sufficient species.

5. Conclusion

Overall, the present study provides new insights into chromosomal evolution in NWMs, thus confirming and extending previous observations. Moreover, our results are bases for more detailed characterization of ECBs in future. The latter may then lead to further investigations of genomic features of ECBs, such as tandem repeats, segmental duplications and copy number variant regions. Meanwhile, our molecular cytogenetic data confirms ideas on involvement of FSs in genomic stability during evolution.

Declarations

Author contribution statement

Xiaobo Fan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Weerayuth Supiwong: Performed the experiments.

http://dx.doi.org/10.1016/j.heliyon.2015.e00042

Anja Weise: Analyzed and interpreted the data; Wrote the paper.

Kristin Mrasek, Nadezda Kosyakova: Analyzed and interpreted the data.

Alongkoad Tanomtong, Krit Pinthong, Vladimir A. Trifonov, Marcelo de Bello Cioffi, Pierre Grothmann, Edivaldo H.C. de Oliveira: Contributed reagents, materials, analysis tools or data.

Thomas Liehr: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Funding statement

Xiaobo Fan was supported by the China Scholarship Council.

Additional information

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.heliyon.2015.e00042.

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