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Exploring copy number variation in the rabbit (*Oryctolagus cuniculus*) genome by array comparative genome hybridization

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ABSTRACT

The European rabbit (*Oryctolagus cuniculus*) is relevant in a large spectrum of fields: it is a livestock, a pet, a biomedical model and a biotechnology tool, a wild resource and a pest. The sequencing of the rabbit genome has opened new perspectives to study this lagomorph at the genome level. We herein investigated for the first time the *O. cuniculus* genome by array comparative genome hybridization (aCGH) and established a first copy number variation (CNV) genome map in this species comprising 155 copy number variation regions (CNVRs; 95 gains, 59 losses, 1 with both gain and loss) covering ~0.3% of the OryCun2.0 version. About 50% of the 155 CNVRs identified spanned 139 different protein coding genes, 110 genes of which were annotated or partially annotated (including Major Histocompatibility Complex genes) with 277 different gene ontology terms. Many rabbit CNVRs might have a functional relevance that should be further investigated.

1. Introduction

The economic importance of the European rabbit (*Oryctolagus cuniculus*) is probably underestimated: the rabbit is a livestok, it produces meat, fur and wool; it is a pet and a fancy animal with many different breeds/lines with a broad variety of different phenotypic characteristics; it is a biomedical model and a biotechnology tool used to answer many biological questions and to produce biomedical reagents; and it is a wild resource and a pest in several regions in which it has been introduced. Despite the large spectrum of fields in which the rabbit is relevant, relatively few studies, compared to other species, have investigated its genome. In particular, reciprocal chromosome painting between human and rabbit was used to establish homology chromosome maps for these two species [1]. Subsequently, a first whole genome integrated genetic and cytogenetic microsatellite map has been obtained and refined by Chantry-Darmon et al. [2–4]. Then the Broad Institute sequenced the rabbit genome within the Mammalian Genome Project whose aim was to obtain comparative sequencing data useful to annotate the human genome [5]. A preliminary low coverage version (2X) of the rabbit genome has been recently improved and reassembled at ~7X (OryCun2.0 version; http://www.ensembl.org/Oryctolagus_cuniculus/Info/Index). In rabbits only a few reports have characterized the genetic basis of phenotypic traits, like coat colour [6–9] and hair morphology [4,10], or have investigated quantitative trait loci for biomedical and production traits [11–13].

Recently, a large number of studies have demonstrated that copy number variation (CNV), defined as intraspecific gains or losses of ≥1 kb of genomic DNA, is quite frequent in the mammalian genomes in which it represents the largest source of variability in terms of affected nucleotides (~0.4–25% of the genome; i.e. [14,15]). Therefore, it is not surprising that CNVs can regulate gene expression and functions (i.e. [16] and that are involved in many human Mendelian and complex disorders [17] and many other phenotypic traits in other species (i.e. [18–20]). Among domesticated animals, CNV maps have been produced in dog [21,22], cattle [23–25], sheep [26], goat [27], pig [28,29], and chicken [30] using array comparative genome hybridization (aCGH) or high density SNP chips [31,32].

We herein investigated for the first time the *O. cuniculus* genome by aCGH in order to identify CNVs and establish a first CNV genome map in this species.

2. Results and discussion

2.1. Validation of aCGH data

Our previous aCGH studies in sheep and goats were based on a heterologous genome (cattle) used to design the tiling arrays applied to investigate CNV in these two close small ruminant species [26,27], as their genomes were not assembled at that time. In the current study we

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used the assembled rabbit genome (OryCun2.0) to analyze CNV in genomic DNA samples coming from the same species. Arrays that contained ~760,000 probes with an average interval of ~3 kb were used to analyze three target DNA samples from rabbits of different lines/breeds (one Commercial white line doe, one Checkered Giant doe and one Champagne d'Argent buck) chosen to maximize possibilities to detect variability, against a reference DNA from an inbred Rhinelander doe. These homologous aCGH data were analyzed using the three better performing algorithms for segmentation analysis implemented in the CGHweb package (see Materials and methods [33]), averaging these results to obtain a suitable $\log_2$ ratio threshold. As in this experimental design we included two different controls, we empirically detected the $\log_2$ ratio threshold based on them: 1) in the self-self hybridization analysis the threshold should not obtain any CNV call (control of false positive results); 2) in the hybridization having as target genome that of the Champagne d'Argent male (hybridized with a reference female genomic DNA sample), $\log_2$ ratio was set up to detect differences of copy number (loss) on more than 98% of the X chromosome (control of false negative results; Fig. 1). Based on these criteria the $\log_2$ ratio threshold to call gains and losses was empirically established at 0.4. Applying this threshold we could reduce the problems of false positive and false negative calls even if, in this evaluation, we could not fully control the differences of false positive/negative rates across arrays.

In addition, three CNVRs (see below data about all CNVRs detected in this study), identified on *O. cuniculus* chromosomes (OCU) 5, 13, and 17 were validated by semiquantitative fluorescent multiplex-PCR (SQF-PCR; Table 1 and Table S1). Two consisted of gains (OCU5 and OCU17) and one of loss (OCU13), as determined in the aCGH experiments. Considering the results obtained for the three animals used in the aCGH analyses and the three investigated CNVRs, all SQF-PCR assays, except one, confirmed results obtained in the aCGH experiments (8 out of 9). Only for the region on OCU13, SQF-PCR in the Commercial white line rabbit did not indicate a loss of copies compared to the reference Rhinelander genomic DNA, as it was obtained from the aCGH experiment (Table S1). Based on our limited validation results, the false positive rate is 1/9 or ~11%. This evaluation is close to the rate of non validated aCGH data reported in cattle using qPCR (~8% [24]), whereas is far lower than the non validated data reported in a pig aCGH study (50%) based on a preliminary assembly of a few porcine chromosomes [27].

SQF-PCR results produced from additional animals not used in the aCGH experiments in order to further investigate these regions indicated

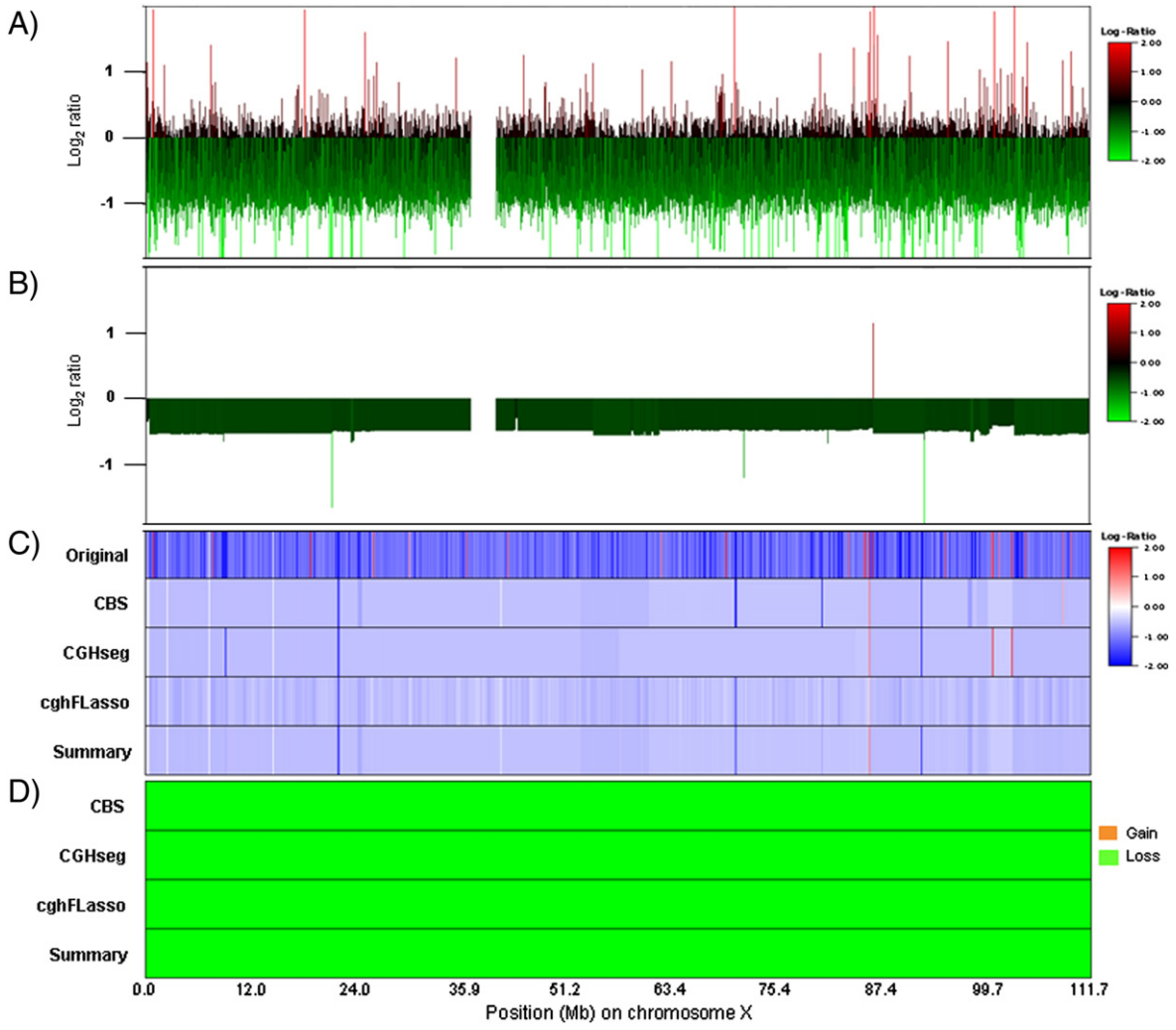

Fig. 1. Results of the aCGH for the X chromosome in the male Champagne d'Argent rabbit. Images have been reported for A) $\log_2$ ratio plot of original data, B) $\log_2$ ratio plot of summary data (pointwise averaging of all computed profiles), C) heatmap of $\log_2$ ratios for original, smoothed/segmented, and summary data, and D) maps of gains/losses for smoothed/segmented and summary data (gain is indicated in orange, loss is indicated in green). Smoothed/segmented data were obtained with three algorithms (CBS, CGHseq, and cghFLasso; see also Materials and methods) averaged in the summary data.

Table 1
Validated CNVRs using semiquantitative fluorescent multiplex-PCR (SQF-PCR).

CNVR no.[a]	Chromosome (OCU)	OCU coordinates [b]	Target gene symbol (Ensembl entry no.)	Gain/loss in aCGH [c]	Gain/loss in SQF-PCR [d]
56	5	24,035,242–24,061,529	–	Gain	Gain/loss[e]
112	13	131,093,402–131,135,020	–	Loss	Loss
129	17	31,127,859–31,165,791	*DLL4* (ENSOCUG00000010756), *CHAC1* (ENSOCUG00000010762)	Gain	Gain

[a] As listed in Supplementary file 2.
[b] OCU coordinates on the OryCun2.0 genome version of the corresponding CNVR.
[c] Results obtained in the aCGH assays.
[d] Results obtained in the SQF-PCR assays.
[e] Loss of copies reported for other rabbits not used in the aCGH experiment (see Table S1).

that the OCU5 CNVR included both gain and loss of copies, and that gain of copies for the OCU17 CNVR was present in additional rabbits (Table S1).

2.2. CNVs in the rabbit genome

Using the stringent and conservative approach we applied in calling CNV from the aCGH experiments, we reported a total of 196 CNVs (Table 2) with an average size of 38.5 kb (ranging from 11.6 kb to 364.4 kb; Supplementary file 1). This is about half the average size observed in the sheep and goat aCGH analyses, that were carried out with about half the number of probes (~385,000) in the chips [26,27], that means that higher is the density of the aCGH platform, higher is the potential resolution power, that, of course, depends also from the criteria used to call CNVs. For example, in this study we considered only CNVs detected by at least 5 consecutive probes. The White commercial rabbit showed the highest number of CNVs (88), most of which were unique (observed only in that animal; 0.75) and consisting of gains (~0.70; Table 2). Champagne d'Argent male showed the lowest number of CNVs (47) that were gains or losses in almost equal proportion (~0.50). Fig. 2 reports a few examples of CNVs identified in the analyzed rabbit using the CGHweb package.

CNVRs were determined by aggregating overlapping CNVs identified in different rabbits using criteria already used in other studies [14,24,26,27]. On the whole we identified 155 CNVRs covering 6.62 Mb (~0.3%) of the OryCun2.0 genome version anchored to the 21 autosomes and the X chromosome (Fig. 3 and Supplementary file 2). The unassembled scaffolds (about 18% of the sequenced genome) were not included in this first investigation due to problems in interpreting the results [24,26]. These 155 CNVRs included 95 gains, 59 losses and one with both gain and loss compared to the reference sample, localized on all chromosomes except on OCU20 (Fig. 3 and Supplementary file 2). Chromosomes with more than ten CNVRs were OCU1, OCU2, OCU3, OCU4 and OCU13 (11, 11, 13, 14, and 14, respectively), whereas OCU5 and OCU4 were the most covered by CNVRs (1.087% and 0.820%, respectively; Table S2). Seventeen CNVRs were identified in more than one rabbit (11%) and were defined as high confidence CNVRs, whereas the remaining 89% were considered as second level confidence CNVRs as previously defined [26]. Mean and median of these 155 CNVRs were 42.7 kb and 23.4 kb, respectively (ranging from ~11.7 to ~346.4 kb). Other aCGH studies in domestic animals used a lower density array (~385,000 probes; [21,24,26,27,30]) or higher density arrays (i.e. [23]) and for these reasons it is possible to have only an indirect comparison between our results and those obtained in other species. For example, the CNVR average size in experiments using about half the number of probes than in our study was almost twice the size that we report here in rabbit, whereas the number of CNV called in each analyzed animal was 2-3 times lower than what we identified in our explorative investigation in *O. cuniculus* [21,24,26,27,30].

2.3. Annotation of CNVRs

About 50% (78) of the 155 CNVRs identified in the rabbit genome partially or completely spanned 139 different protein coding genes, 110 genes of which were annotated or partially annotated with 277 different Gene Ontology (GO) terms (Supplementary file 3 and data not shown). A few of these categories were significantly or suggestively (from $P<0.01$ to $P<0.1$) over-represented in rabbit CNVRs (Table 3). Similarly to what we obtained in sheep, this study in rabbit reported fewer over-represented groups compared to other CNV studies [21,22,27,34]. This might be due to a bias derived by the reference chosen in this experiment and/or by the different number of analyzed animals compared to the other experiments. Even considering these limits, it is particularly interesting to note that antigen binding, antigen processing and presentation were the GO over-represented GO terms of the "Molecular functions" and "Biological Process" categories respectively (Table 3). In the "Cellular component" classification, MHC protein complexes and plasma membrane part were over-represented categories in rabbit CNVRs (Supplementary file 3). The Major Histocompatibility Complex (MHC) of the rabbit appeared affected by copy number polymorphisms. In particular, CNVRs 90 and 93 on OCU12 contained MHC class I and class II genes, respectively (Supplementary file 3). The MHC class I and II genes belong to gene families that include functional genes as well as pseudogenes [35]. The class I molecules are known to present self-antigens to cytotoxic CD8+ T lymphocytes and regulate Natural Killer cell activity and the class II molecules present exogeneous peptides to CD4+ T lymphocytes. Both class I and II genes are highly polymorphic, mostly in the regions encoding the peptide groove. Direct investigations of MHC in other species, including pig and primates, reported similar variability in class I and class II genes [36–38]. In addition, other aCGH studies have already shown that MHC genes in ruminants are affected by CNV [23,24,26,27]. All these studies indicated that CNV is an additional common source of variability in the MHC and differences in copy number might be important to define disease resistance or

Table 2
Summary of CNVs identified in the analyzed rabbits.

Rabbits	Number of CNVs				CNV average size ± s.d. (in kb)
	Total	Unique[a]	Gain	Loss	
Champagne d'Argent (male)	47[b]	42	24	23	38.9 ± 47.2
Checkered Giant (female)	61	45	39	22	37.3 ± 55.8
Commercial white line (female)	88	66	62	26	36.6 ± 46.9
Total	196	153	125	71	38.5 ± 46.9

[a] Not overlapping with any other CNV.
[b] Excluding chromosome X data.

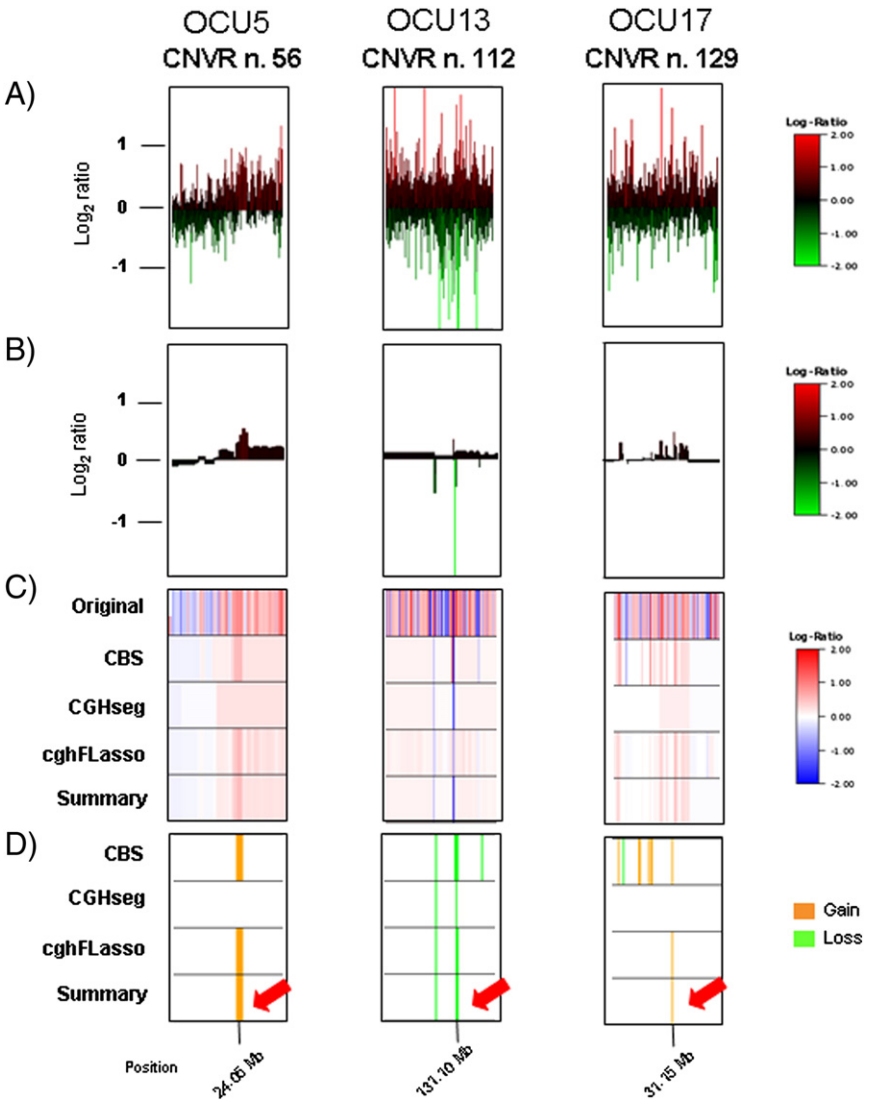

Fig. 2. Examples of three CNVRs in different rabbits and chromosomes (OCU5, OCU13 and OCU17) identified by aCGH. Images have been reported as described in the legend of Fig. 1. Red arrows indicate regions of copy gain or loss.

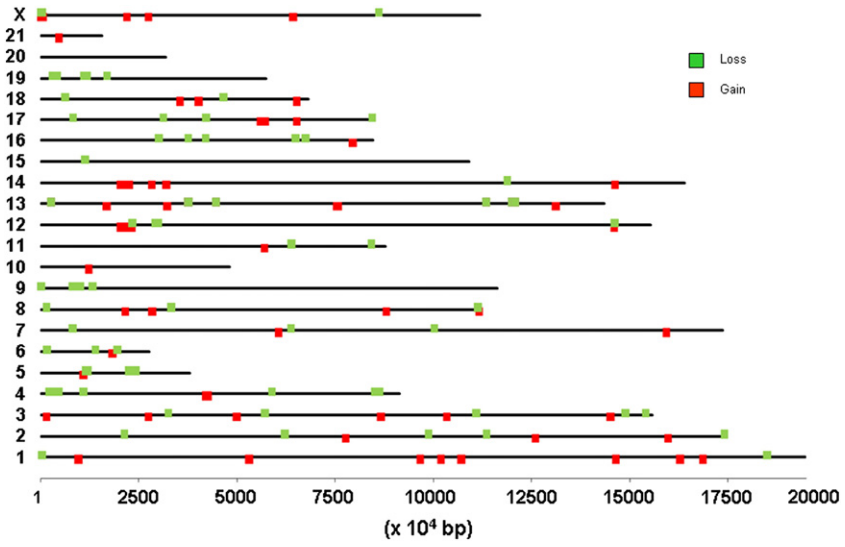

Fig. 3. Map of rabbit CNVRs identified in this study (reported in the different rabbit chromosomes).

Table 3
Gene ontology (GO) categories significantly overrepresented in rabbit copy number variation regions (CNVRs).

GO level[a]	GO term	GO name	FDR[b]	No. in rabbit CNVRs	Expected number
Molecular function	GO:0003823	Antigen binding	<0.005	4	0.001
Biological process	GO:0019882	Antigen processing and presentation	<0.01	5	0.005
Cellular component	GO:0042611	MHC protein complex	<0.005	5	0.003
Cellular component	GO:0042612	MHC class I protein complex	<0.05	3	0.002
Cellular component	GO:0042613	MHC class II protein complex	<0.1	2	0.001
Cellular component	GO:0044459	Plasma membrane part	<0.1	12	0.08

[a] Analyses are referred to the GO annotation of the rabbit genome (OryCun2.0): 57, 57, and 59 transcripts in rabbit CNVRs out of 110 are endowed with a GO annotation for molecular function, biological process and cellular component, respectively; 13,916, 11,156, and 10,763 transcripts in the rabbit genome out of 28,188 transcripts are endowed with a GO annotation for molecular function, biological process and cellular component, respectively.
[b] False discovery rate.

susceptibility in animals. It will be critical to assess whether the CNVs identified in the rabbit MHC affect functional genes.

Among the many other CNVRs with annotated genes, it is interesting to mention CNVR n. 62 on OCU7 that includes an olfactory receptor gene (olfactory receptor, family 2, subfamily A, member 14; *OR2A14*) that is affected by CNV also in human [17,39]. Extensive CNV in olfactory receptor gene families has been reported within and among human and mouse populations and might be important to define olfactory inter-individual differences [34,39,40]. Other four CNVRs on OCU13 (n. 102, 104, 107, and 110) encompass genes (dolichyl-phosphate mannosyltransferase polypeptide 3, *DPM3*; vang-like 1 (van gogh, *Drosophila*), *VANGL1*; low density lipoprotein receptor-related protein 8, apolipoprotein e receptor, *LRP8*; and fatty acid amide hydrolase, *FAAH*) that are responsible of human genetic defects (congenital disorder of glycosylation 1o; caudal regression syndrome, and neural tube defects; susceptibility to myocardial infarction 1; and susceptibility to drug addiction; respectively) as reported in OMIM database (January 2012). Moreover, CNVR n. 85 on OCU11 encompasses the sperm flagellar 2 (*SPEF2*) gene, also known as *KPL2*, that in pig an intronic insertion causes the immotile short-tail sperm defect [41].

CNV has been recently identified in the rabbit alpha globin gene. Unfortunately this gene is not correctly assembled in the OryCun2.0 genome version and for this reason we could have missed it from our study even if it is still not clear if this variability is present only in wild rabbit populations or also in domesticated populations [42].

3. Conclusions

The sequencing of the rabbit genome has opened new opportunities to exploit genomic information of this multifaceted species that is important in many basic and applied biological fields. Despite the progress that will be derived by this initiative, many other studies should be carried out to refine this resource, improving its assembly and annotation, and to characterize its variability at different levels. In this study we have explored copy number variability in the rabbit genome by aCGH using the OryCun2.0 genome version to design the tiling arrays, and using strict calling criteria we and reported 155 CNVRs. As in this first trial we have analyzed a limited number of rabbits, the identified CNVRs represent only a small fraction of this kind of variability in the *O. cuniculus* genome. A larger number of animals of different populations and breeds should be analyzed to have a more complete picture of CNV distribution and characteristics in this lagomorph species. However, with the current genome version more challenging investigations will have as drawback the limits derived by the quite high fraction of not assigned contigs, and by the well known problems that preliminary genome versions might have in terms of incomplete sequenced regions and contig orientations that might be critical to study, for example, relationships between segmental duplications and CNV (as already reported in many other species; i.e. [14]) or between CNV and gene expression level. Even with the limited number of analyzed rabbits, interesting results were obtained. Several CNVs include genes already shown to have important biological functions. Therefore, copy number polymorphisms affecting them might explain a quote of variability of phenotypic and production traits of different breeds and lines selected for different purposes. In addition, immunological aspects of this species might be influenced, at least in part, by CNV that might also be important to investigate considering the rabbit as a biomedical model and as a biotechnology tool.

4. Materials and methods

4.1. aCGH analyses

Array comparative genome hybridization experiments were based on a customized tiling array designed by Roche NimbleGen Inc. (Madison, WI; http://www.nimblegen.com) on the *O. cuniculus* genome, OryCun2.0 version (http://www.ensembl.org/Oryctolagus_cuniculus/Info/Index). Arrays contained ~760,000 probes on a single slide to provide an evenly distributed coverage with an average interval of ~3 kb for the OryCun2.0 genome. Rabbit genomic DNA was extracted from blood of one doe of a Commercial white line (CWL) of Gruppo Martini [43], one Checkered Giant doe (CG1), one Champagne d'Argent buck (CdA), and one Rhinelander (Tricolor; R1) doe using the Wizard® Genomic DNA Purification kit (Promega Corporation,Madison, WI). Reference DNA was from the Rhinelander doe. This DNA was labeled with Cy5 and co-hybridized with the other three test DNA samples labeled with Cy3 on 3 different arrays. A self hybridization (reference DNA labeled by both Cy5 and Cy3) was carried out in another array. Hybridization and array scanning were performed by Roche NimbleGen as previously described [34]. Data normalization was conducted using the normalize.qsline method from the Bioconductor package in R [34]. Then data were analyzed for each hybridization using normalized $\log_2$ ratios using the CGHweb R package for Linux (http://compbio.med.harvard.edu/CGHweb/ [33]). Pointwise averaging of all computed profiles and maps of gains/losses for smoothed/segmented obtained from three algorithms implemented in this package (Circular Binary Segmentation [44]; Gaussian Model with Adaptive Penalty [45]; cghFLasso [46]), shown to have better performances ([33]; and data not shown) were generated. We used the self-self hybridization and the chromosome X data of the Champagne d'Argent rabbit used as control region to define a suitable threshold to apply to the CGHweb calls in order to minimize false positive and false negative results. The Champagne d'Argent male is expected to have one copy of the X chromosome compared to the reference Rhinelander doe (loss). We detected loss over at least 98% of the X chromosome in this male/female comparison. Summary data obtained considering the three indicated algorithms were generated to call gain/loss in a chromosome region and to compile a high confidence set of CNVs. Then CNVs were called considering a conservative approach joining regions of at least 5 contiguous probes with CNV signal separated by up to two probes without CNV signal in the same individual. Copy number variation regions (CNVRs) were reported aggregating overlapping or partially overlapping CNVs in different animals as previously reported [14,24,26,27] and applying the same criteria for CNVs within individuals.

4.2. Validation of CNVs by semiquantitative fluorescent multiplex PCR

Semiquantitative fluorescent multiplex PCR (SQF-PCR) was used to validate several CNVs identified in the aCGH experiments (Table S1). This technique was applied as previously described [19,26,27,47] using genomic DNA of the same rabbits analyzed in the aCGH experiments and genomic DNA of other five rabbits (one Rhinelander, R2; and four Checkered Giant does, CG2-5 of a reference families developed by Fontanesi et al. [48] extracted as described above. Briefly, one internal control region known to have no CNV (myostatin, *MSTN* [43]) and CNVR of interest were co-amplified in multiplex PCR under quantitative PCR conditions (with forward primers labeled in 5′ with 6FAM) and the products were separated by capillary electrophoresis using an ABI3100 Avant sequencer (Applied Biosystems, Foster City, CA, USA; [19]). Peak heights of regions of interest were normalized against those of the internal control. PCR primers and PCR conditions are reported in Table S1. Capillary electrophoresis was performed using 1 μL of reaction product, diluted in 10 μL of Hi-Di formamide (Applied Biosystems), and added with 0.1 μL of Rox labelled DNA ladder (500HD Rox, Applied Biosystems). Peak heights were obtained using GeneScan software v. 3.7 (Applied Biosystems). DNA dosages were calculated by comparing the normalized peak height ratios of rabbits of interest with the average normalized ratios of the reference Rhinelander rabbit as previously described ([19,26,27]; see also Table S1). At least three analyses were carried out for each sample/primer pair combination, and average results and standard deviation were calculated. This method was applied to validate the results obtained with aCGH and not to precisely estimate the number of copies of the analyzed DNA fragments.

4.3. Bioinformatics analyses

Rabbit CNVRs superimposing with transcripts annotated in the OryCun2.0 version were determined on the basis of the genome coordinates, without imposing a minimum overlap threshold. Gene ontology terms associated with rabbit transcripts were downloaded with the Ensembl BioMart retrieval system (http://www.ensembl.org/biomart/index.html) and the complete annotation was obtained by reconstructing the complete list of ancestors of each term in the directed acyclic graph described by the OBO file downloaded from the Gene Ontology web site on November 2011 (http://www.geneontology.org/). The GOTermFinder tool was adopted for this task (http://search.cpan.org/dist/GO-TermFinder/). We computed the occurrence of each term in the set of transcripts overlapping with rabbit CNVRs and we compared it with the occurrence of the same term in the whole rabbit genome (OryCun 2.0). The Fisher exact test was adopted to assess the significance of the overrepresentation of the terms in the set of transcripts overlapping with the rabbit CNVRs. The multiple-hypothesis correction was adopted for discriminating the significant terms at different False Discovery Rates [49]: 0.005, 0.01, 0.05, and 0.1. aCGH data and results have been submitted to the ArrayExpress Archive (http://www.ebi.ac.uk/arrayexpress/), under the accession number E-MEXP-3636.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygeno.2012.07.001.

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References

[1] R. Korstanje, P.C. O'Brien, F. Yang, W. Rens, A.A. Bosma, H.A. van Lith, L.F. van Zutphen, M.A. Ferguson-Smith, Complete homology maps of the rabbit (*Oryctolagus cuniculus*) and human by reciprocal chromosome painting, Cytogenet. Cell Genet. 86 (1999) 317–322.
[2] C. Chantry-Darmon, C. Rogel-Gaillard, M. Bertaud, C. Urien, M. Perrocheau, P. Chardon, H. Hayes, 133 new gene localizations on the rabbit cytogenetic map, Cytogenet. Genome Res. 103 (2003) 192–201.
[3] C. Chantry-Darmon, C. Urien, H. Hayes, M. Bertaud, S. Chadi-Taourit, P. Chardon, D. Vaiman, C. Rogel-Gaillard, Construction of a cytogenetically anchored-microsatellite map in rabbit, Mamm. Genome 16 (2005) 442–459.
[4] C. Chantry-Darmon, C. Urien, H. Rochambeau, D. Allain, B. Pena, H. Hayes, C. Grohs, E.P. Cribiu, S. Deretz-Picoulet, C. Larzul, J.C. Save, A. Neau, P. Chardon, C. Rogel-Gaillard, A first generation microsatellite-based integrated genetic and cytogenetic map for the European rabbit (*Oryctolagus cuniculus*) and localization of *angora* and *albino*, Anim. Genet. 37 (2006) 335–341.
[5] K. Lindblad-Toh, M. Garber, O. Zuk, M.F. Lin, B.J. Parker, S. Washietl, P. Kheradpour, J. Ernst, G. Jordan, E. Mauceli, L.D. Ward, C.B. Lowe, A.K. Holloway, M. Clamp, S. Gnerre, J. Alföldi, K. Beal, J. Chang, H. Clawson, J. Cuff, F. Di Palma, S. Fitzgerald, P. Flicek, M. Guttman, M.J. Hubisz, D.B. Jaffe, I. Jungreis, W.J. Kent, D. Kostka, M. Lara, A.L. Martins, T. Massingham, I. Moltke, B.J. Raney, M.D. Rasmussen, J. Robinson, A. Stark, A.J. Vilella, J. Wen, X. Xie, M.C. Zody, Broad Institute Sequencing Platform, Whole Genome Assembly Team, J. Baldwin, T. Bloom, C.W. Chin, D. Heiman, R. Nicol, C. Nusbaum, S. Young, J. Wilkinson, K.C. Worley, C.L. Kovar, D.M. Muzny, R.A. Gibbs, Baylor College of Medicine Human Genome Sequencing Center Sequencing Team, A. Cree, H.H. Dihn, G. Fowler, S. Jhangiani, V. Joshi, S. Lee, L.R. Lewis, L.V. Nazareth, G. Okwuonu, J. Santibanez, W.C. Warren, E.R. Mardis, G.M. Weinstock, R.K. Wilson, Genome Institute at Washington University, K. Delehaunty, D. Dooling, C. Fronik, L. Fulton, B. Fulton, T. Graves, P. Minx, E. Sodergren, E. Birney, E.H. Margulies, J. Herrero, E.D. Green, D. Haussler, A. Siepel, N. Goldman, K.S. Pollard, J.S. Pedersen, E.S. Lander, M. Kellis, A high-resolution map of human evolutionary constraint using 29 mammals, Nature 478 (2011) 476–482.
[6] B. Aigner, U. Besenfelder, M. Müller, G. Brem, Tyrosinase gene variants in different rabbit strains, Mamm. Genome 11 (2000) 700–702.
[7] L. Fontanesi, M. Tazzoli, F. Beretti, V. Russo, Mutations in the melanocortin 1 receptor (*MC1R*) gene are associated with coat colours in the domestic rabbit (*Oryctolagus cuniculus*), Anim. Genet. 37 (2006) 489–493.
[8] L. Fontanesi, L. Forestier, D. Allain, E. Scotti, F. Beretti, S. Deretz-Picoulet, E. Pecchioli, C. Vernesi, T.J. Robinson, J.L. Malaney, V. Russo, A. Oulmouden, Characterization of the rabbit agouti signaling protein (*ASIP*) gene: transcripts and phylogenetic analyses and identification of the causative mutation of the nonagouti black coat colour, Genomics 95 (2010) 166–175.
[9] L. Fontanesi, E. Scotti, M. Colombo, F. Beretti, L. Forestier, S. Dall'Olio, S. Deretz, V. Russo, D. Allain, A. Oulmouden, A composite six bp in-frame deletion in the melanocortin 1 receptor (*MC1R*) gene is associated with the Japanese brindling coat colour in rabbits (*Oryctolagus cuniculus*), BMC Genet. 11 (2010) 59.
[10] M. Diribarne, X. Mata, C. Chantry-Darmon, A. Vaiman, G. Auvinet, S. Bouet, S. Deretz, E.P. Cribiu, H. de Rochambeau, D. Allain, G. Guérin, A deletion in exon 9 of the *LIPH* gene is responsible for the rex hair coat phenotype in rabbits (*Oryctolagus cuniculus*), PLoS One 6 (2011) e19281.
[11] R. Korstanje, G.F. Gillissen, L.P. Kodde, M. Den Bieman, A. Lankhorst, L.F. Van Zutphen, H.A. Van Lith, Mapping of microsatellite loci and association of aorta atherosclerosis with LG VI markers in the rabbit, Physiol. Genomics 6 (2001) 11–18.
[12] W.A. van Haeringen, M. Den Bieman, G.F. Gillissen, A.E. Lankhorst, M.T. Kuiper, L.F. van Zutphen, H.A. van Lith, Mapping of a QTL for serum HDL cholesterol in the rabbit using AFLP technology, J. Hered. 92 (2001) 322–326.
[13] I. Sternstein, D. Maj, J. Bieniek, G.A. Brockmann, A new linkage map of the rabbit (*Oryctolagus cuniculus*) chromosome 1 (*OCU1*) and results of a QTL analysis for carcass composition and meat quality traits, In: Proceedings of the 9th World Congress on Genetics Applied to Livestock Production, 2010, communication no. 0425.
[14] R. Redon, S. Ishikawa, K.R. Fitch, L. Feuk, G.H. Perry, T.D. Andrews, H. Fiegler, M.H. Shapero, A.R. Carson, W. Chen, E.K. Cho, S. Dallaire, J.L. Freeman, J.R. González, M. Gratacòs, J. Huang, D. Kalaitzopoulos, D. Komura, J.R. MacDonald, C.R. Marshall, R. Mei, L. Montgomery, K. Nishimura, K. Okamura, F. Shen, M.J. Somerville, J. Tchinda, A. Valsesia, C. Woodwark, F. Yang, J. Zhang, T. Zerjal, J. Zhang, L. Armengol, D.F. Conrad, X. Estivill, C. Tyler-Smith, N.P. Carter, H. Aburatani, C. Lee, K.W. Jones, S.W. Scherer, M.E. Hurles, Global variation in copy number in the human genome, Nature 444 (2006) 444–454.
[15] D.F. Conrad, D. Pinto, R. Redon, L. Feuk, O. Gokcumen, Y. Zhang, J. Aerts, T.D. Andrews, C. Barnes, P. Campbell, T. Fitzgerald, M. Hu, C.H. Ihm, K. Kristiansson, D.G. Macarthur, J.R. Macdonald, I. Onyiah, A.W. Pang, S. Robson, K. Stirrups, A. Valsesia, K. Walter, J. Wei, Wellcome Trust Case Control Consortium, C. Tyler-Smith, N.P. Carter, C. Lee, S.W. Scherer, M.E. Hurles, Origins and functional impact of copy number variation in the human genome, Nature 464 (2010) 704–712.
[16] B.E. Stranger, M.S. Forrest, M. Dunning, C.E. Ingle, C. Beazley, N. Thorne, R. Redon, C.P. Bird, A. de Grassi, C. Lee, C. Tyler-Smith, N. Carter, S.W. Scherer, S. Tavaré, P. Deloukas, M.E. Hurles, E.T. Dermitzakis, Relative impact of nucleotide and copy number variation on gene expression phenotypes, Science 315 (2007) 848–853.
[17] P. Stankiewicz, J.R. Lupski, Structural variation in the human genome and its role in disease, Annu. Rev. Med. 61 (2010) 437–455.
[18] B.J. Norris, V.A. Whan, A gene duplication affecting expression of the ovine *ASIP* gene is responsible for white and black sheep, Genome Res. 18 (2008) 1282–1293.

[19] L. Fontanesi, F. Beretti, V. Riggio, E. Gómez González, S. Dall'Olio, R. Davoli, V. Russo, B. Portolano, Copy number variation and missense mutations of the agouti signaling protein (*ASIP*) gene in goat breeds with different coat colors, Cytogenet. Genome Res. 126 (2009) 333–347.
[20] D. Wright, H. Boije, J.R. Meadows, B. Bed'hom, D. Gourichon, A. Vieaud, M. Tixier-Boichard, C.J. Rubin, F. Imsland, F. Hallböök, L. Andersson, Copy number variation in intron 1 of *SOX5* causes the Pea-comb phenotype in chickens, PLoS Genet. 5 (2009) e1000512.
[21] W.K. Chen, J.D. Swartz, L.J. Rush, C.E. Alvarez, Mapping DNA structural variation in dogs, Genome Res. 19 (2009) 500–509.
[22] T.J. Nicholas, Z. Cheng, M. Ventura, K. Mealey, E.E. Eichler, J.M. Akey, The genomic architecture of segmental duplications and associated copy number variants in dogs, Genome Res. 19 (2009) 491–499.
[23] J. Fadista, B. Thomsen, L.E. Holm, C. Bendixen, Copy number variation in the bovine genome, BMC Genomics 11 (2010) 284.
[24] G.E. Liu, Y. Hou, B. Zhu, M.F. Cardone, L. Jiang, A. Cellamare, A. Mitra, L.J. Alexander, L.L. Coutinho, M.E. Dell'Aquila, L.C. Gasbarre, G. Lacalandra, R.W. Li, L.K. Matukumalli, D. Nonneman, L.C. Regitano, T.P. Smith, J. Song, T.S. Sonstegard, C.P. Van Tassell, M. Ventura, E.E. Eichler, T.G. McDaneld, J.W. Keele, Analysis of copy number variations among diverse cattle breeds, Genome Res. 20 (2010) 693–703.
[25] J.S. Bae, H.S. Cheong, L.H. Kim, S. NamGung, T.J. Park, J.Y. Chun, J.Y. Kim, C.F. Pasaje, J.S. Lee, H.D. Shin, Identification of copy number variations and common deletion polymorphisms in cattle, BMC Genomics 11 (2010) 232.
[26] L. Fontanesi, F. Beretti, P.L. Martelli, M. Colombo, S. Dall'Olio, M. Occidente, B. Portolano, R. Casadio, D. Matassino, V. Russo, A first comparative map of copy number variations in the sheep genome, Genomics 97 (2011) 158–165.
[27] L. Fontanesi, P.L. Martelli, F. Beretti, V. Riggio, S. Dall'Olio, M. Colombo, R. Casadio, V. Russo, B. Portolano, An initial comparative map of copy number variations in the goat (*Capra hircus*) genome, BMC Genomics 11 (2010) 639.
[28] J. Fadista, M. Nygaard, L.E. Holm, B. Thomsen, C. Bendixen, A snapshot of CNVs in the pig genome, PLoS One 3 (2008) e3916.
[29] Y. Ramayo-Caldas, A. Castelló, R.N. Pena, E. Alves, A. Mercadé, C.A. Souza, A.I. Fernández, M. Perez-Enciso, J.M. Folch, Copy number variation in the porcine genome inferred from a 60 k SNP BeadChip, BMC Genomics 11 (2010) 593.
[30] X. Wang, S. Nahashon, T.K. Feaster, A. Bohannon-Stewart, N. Adefope, An initial map of chromosomal segmental copy number variations in the chicken, BMC Genomics 11 (2010) 351.
[31] N.P. Carter, Methods and strategies for analyzing copy number variation using DNA microarrays, Nat. Genet. 39 (2007) S16–S21.
[32] S.A. McCarroll, F.G. Kuruvilla, J.M. Korn, S. Cawley, J. Nemesh, A. Wysoker, M.H. Shapero, P.I. de Bakker, J.B. Maller, A. Kirby, A.L. Elliott, M. Parkin, E. Hubbell, T. Webster, R. Mei, J. Veitch, P.J. Collins, R. Handsaker, S. Lincoln, M. Nizzari, J. Blume, K.W. Jones, R. Rava, M.J. Daly, S.B. Gabriel, D. Altshuler, Integrated detection and population-genetic analysis of SNPs and copy number variation, Nat. Genet. 40 (2008) 1166–1174.
[33] W. Lai, V. Choudhary, P.J. Park, CGHweb: a tool for comparing DNA copy number segmentations from multiple algorithms, Bioinformatics 24 (2008) 1014–1015.
[34] T.A. Graubert, P. Cahan, D. Edwin, R.R. Selzer, T.A. Richmond, P.S. Eis, W.D. Shannon, X. Li, H.L. McLeod, J.M. Cheverud, T.J. Ley, A high-resolution map of segmental DNA copy number variation in the mouse genome, PLoS Genet. 3 (2007) e3.
[35] R. Horton, L. Wilming, V. Rand, R.C. Lovering, E.A. Bruford, V.K. Khodiyar, M.J. Lush, S. Povey, C.C. Talbot Jr., M.W. Wright, H.M. Wain, J. Trowsdale, A. Ziegler, S. Beck, Gene map of the extended human MHC, Nat. Rev. Genet. 5 (2004) 889–899.
[36] F. Barbisan, C. Savio, G. Bertorelle, T. Patarnello, L. Congiu, Duplication polymorphism at MHC class II DRB1 locus in the wild boar (*Sus scrofa*), Immunogenetics 61 (2009) 145–151.
[37] N.G. de Groot, C.M. Heijmans, N. de Groot, G.G. Doxiadis, N. Otting, R.E. Bontrop, The chimpanzee Mhc-DRB region revisited: gene content, polymorphism, pseudogenes, and transcripts, Mol. Immunol. 4 (2009) 7, 381–7, 389.
[38] M. Tanaka-Matsuda, A. Ando, C. Rogel-Gaillard, P. Chardon, H. Uenishi, Difference in number of loci of swine leukocyte antigen classical class I genes among haplotypes, Genomics 93 (2008) 261–273.
[39] Y. Hasin, T. Olender, M. Khen, C. Gonzaga-Jauregui, P.M. Kim, A.E. Urban, M. Snyder, M.B. Gerstein, D. Lancet, J.O. Korbel, High-resolution copy-number variation map reflects human olfactory receptor diversity and evolution, PLoS Genet. 4 (2008) e1000249.
[40] J.M. Young, R.M. Endicott, S.S. Parghi, M. Walker, J.M. Kidd, B.J. Trask, Extensive copy-number variation of the human olfactory receptor gene family, Am. J. Hum. Genet. 83 (2008) 228–242.
[41] A. Sironen, B. Thomsen, M. Andersson, V. Ahola, J. Vilkki, An intronic insertion in *KPL2* results in aberrant splicing and causes the immotile short-tail sperm defect in the pig, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 5006–5011.
[42] R. Campos, J.F. Storz, N. Ferrand, Copy number polymorphism in the α-globin gene cluster of European rabbit (*Oryctolagus cuniculus*), Heredity 108 (2012) 531–536.
[43] L. Fontanesi, E. Scotti, A. Frabetti, D. Fornasini, A. Piccone, V. Russo, Identification of polymorphisms in the rabbit (*Oryctolagus cuniculus*) myostatin (*MSTN*) gene and association analysis with finishing weight in a commercial rabbit population, Anim. Genet. 42 (2011) 339.
[44] A.B. Olshen, E.S. Venkatraman, R. Lucito, M. Wigler, Circular binary segmentation for the analysis of array-based DNA copy number data, Biostatistics 5 (2004) 557–572.
[45] F. Picard, S. Robin, M. Lavielle, C. Vaisse, J.J. Daudin, A statistical approach for array CGH data analysis, BMC Bioinformatics 6 (2005) 27.
[46] R. Tibshirani, P. Wang, Spatial smoothing and hot spot detection for CGH data using the fused lasso, Biostatistics 9 (2008) 18–29.
[47] C. Le Maréchal, E. Masson, J.M. Chen, F. Morel, P. Ruszniewski, P. Levy, C. Férec, Hereditary pancreatitis caused by triplication of the trypsinogen locus, Nat. Genet. 38 (2006) 1372–1374.
[48] L. Fontanesi, M. Vargiolu, E. Scotti, M. Mazzoni, P. Clavenzani, R. De Giorgio, G. Romeo, V. Russo, Endothelin receptor B (*EDNRB*) is not the causative gene of the *English spotting* locus in the domestic rabbit (*Oryctolagus cuniculus*), Anim. Genet. 41 (2010) 669–670.
[49] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. R. Stat. Soc. Ser. B 57 (1995) 289–300.