

Genome-wide analysis of microsatellite repeats in humans: their abundance and density in specific genomic regions

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Abstract

Background: Simple sequence repeats (SSRs) are found in most organisms, and occupy about 3% of the human genome. Although it is becoming clear that such repeats are important in genomic organization and function and may be associated with disease conditions, their systematic analysis has not been reported. This is the first report examining the distribution and density of simple sequence repeats (1-6 base-pairs (bp)) in the entire human genome.

Results: The densities of SSRs across the human chromosomes were found to be relatively uniform. However, the overall density of SSR was found to be high in chromosome 19. Triplets and hexamers were more predominant in exonic regions compared to intronic and intergenic regions, except for chromosome Y. Comparison of densities of various SSRs revealed that whereas trimers and pentamers showed a similar pattern (500-1,000 bp/Mb) across the chromosomes, di- tetra- and hexa-nucleotide repeats showed patterns of higher (2,000-3,000 bp/Mb) density. Repeats of the same nucleotide were found to be higher than other repeat types. Repeats of A, AT, AC, AAT, AAC, AAG, AGC, AAAC, AAAT, AAAG, AAGG, AGAT predominate, whereas repeats of C, CG, ACT, ACG, AACC, AACG, AACT, AAGC, AAGT, ACCC, ACCG, ACCT, CCCG and CCGG are rare.

Conclusions: The overall SSR density was comparable in all chromosomes. The density of different repeats, however, showed significant variation. Tri- and hexa-nucleotide repeats are more abundant in exons, whereas other repeats are more abundant in non-coding regions.

Background

Microsatellites or simple sequence repeats (SSRs) are tandemly repeated DNA sequences found in varying abundance in most genomes [1,2]. These repeats have been extensively used for genetic mapping and population studies [3]. SSRs also provide molecular tools to understand spatial relationships between chromosome segments, which in turn, aid in analyzing temporal relationships between species and genera [4]. On the evolutionary timescale SSRs are dynamic,

as they undergo replication slippage, a mutation event that aids in their expansion or contraction. It is also suggested that SSRs undergo a life cycle - they are born, they grow and finally they die. The entire life cycle of an SSR may span tens or even hundreds of millions of years [5,6]. A growing number of neurological disorders are found to be the consequence of the expansion of a particular class of repeats, the trinucleotide repeats [7-9]. In humans about 3% of the genome is occupied by SSRs [10].

SSRs are distributed throughout the genome in both coding and non-coding regions [1]. Certain repeats are preferred and are often predominant in certain genomic locations. However, the significance of this observation is unclear. Triplets predominate in coding regions [11]. The study of repeat density and its distribution pattern in the genome is expected to help in understanding their significance. There is accumulating evidence to suggest that SSRs function to regulate gene expression [12,13].

The availability of complete genome sequences for many organisms has made it possible to carry out genome-wide analyses. In the present study we have screened the entire human genome to study the distribution and density of microsatellite (1-6 bp) repeats.

Results

We have analyzed the distribution of perfect SSRs spanning 12 bp or more in the complete human genome. Thus, for a 12 bp SSR, one occurrence may comprise a repeat of 12 monomers, or six dimers, or four trimers, or three tetramers (or pentamers) or two hexamers. The SSR data presented here includes both strands of the DNA sequence. AGAT, for

example, also includes GATA and the reverse complements TATC and CTAT, and all possible non-overlapping base combinations. Analyzed sequences were classified into three genomic regions, namely exons (including untranslated regions (UTRs)), introns and intergenic regions. For this analysis, we calculated the total lengths of all mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide repeats in terms of base pairs of SSR per megabase pair (Mb) of DNA.

Abundance of SSRs in the human genome

We have determined the total number of loci for all the SSRs in the human genome on a chromosome-by chromosome basis, and then calculated the number of repeat units at each locus. As expected, the total number of occurrences of SSRs depends on the size of the chromosome. For example, chromosome 1 has the highest and Y the lowest SSR occurrence. We then analyzed density of each repeat as mentioned in Materials and methods. We show that the overall SSR density is comparable across chromosomes (Figure 1). However, some chromosomes, such as 16, 17, 19 and 22, showed a slight increase in SSR density. The highest SSR density was found in chromosome 19 (20,351 bp/Mb) followed by 17 (17,385 bp/Mb) and 22 (16,147 bp/Mb). It is interesting to note that the Y chromosome, although

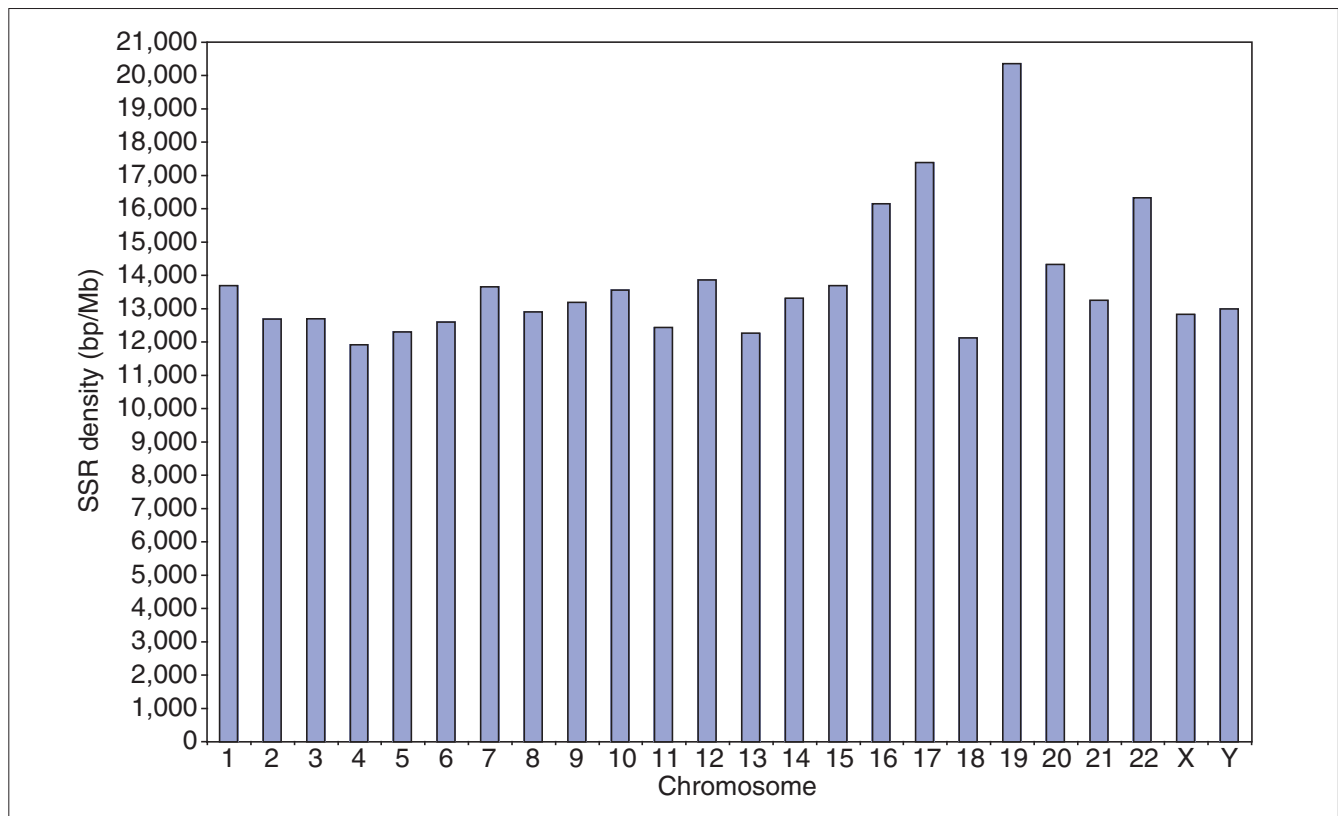


Figure 1
Overall SSR density across the human chromosome set. The density is expressed in base-pairs of SSR sequence per megabase-pairs of chromosome sequence.

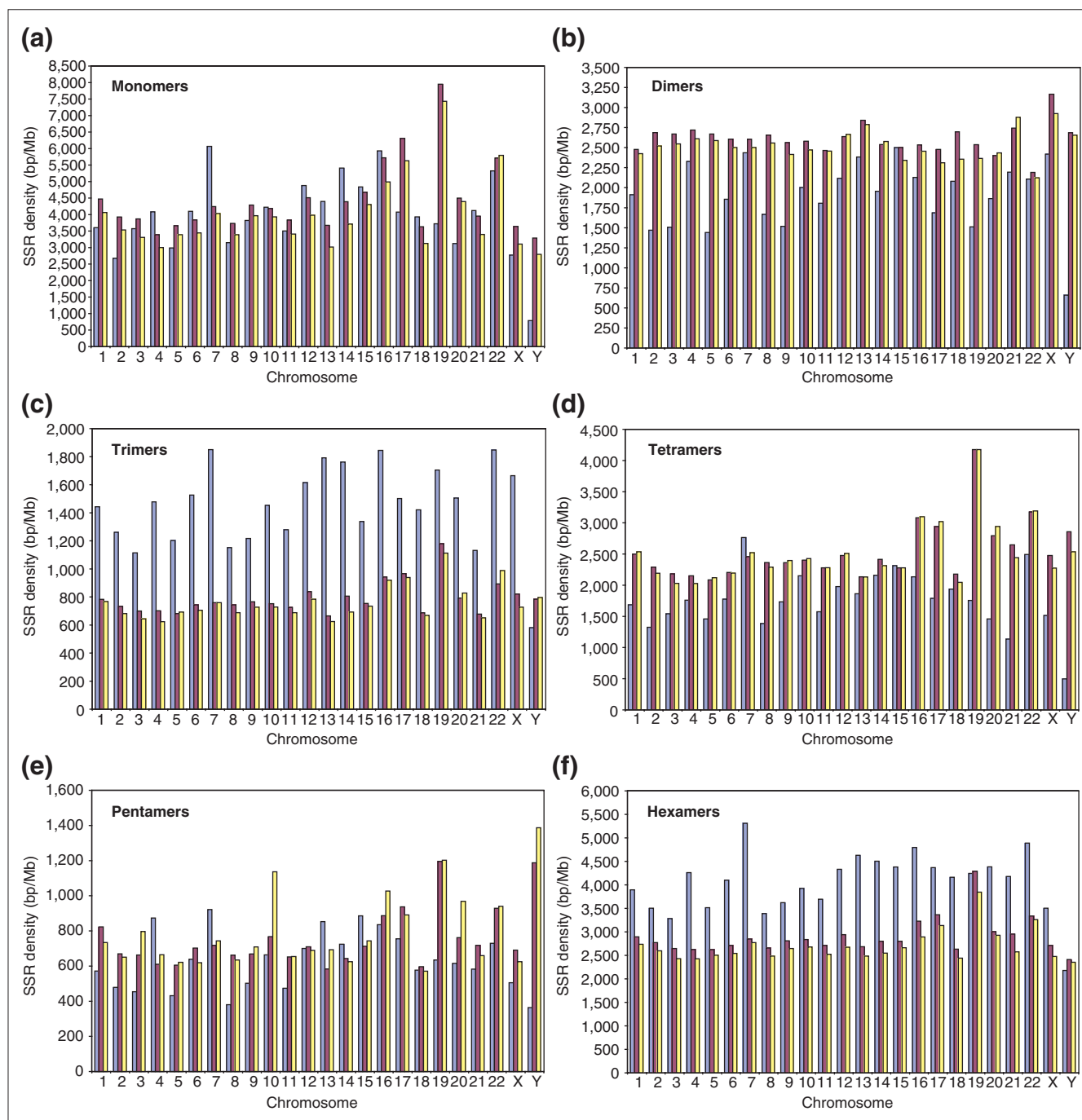


Figure 2 SSR density in exonic, intronic and intergenic regions on individual human chromosomes. **(a)** Monomers **(b)** dimers; **(c)** trimers; **(d)** tetramers; **(e)** pentamers; **(f)** hexamers. Blue bars, exons; red bars, introns; yellow bars, intergenic regions.

showing the smallest number of SSRs, has an SSR density comparable to other autosomes and the X chromosome (Figure 1).

We have carried out a comprehensive analysis of each SSR found in the human genome, which is available as additional

data available with the online version of this paper (see additional data files). For a given repeat, the number of repeats and total length of the repeated sequence are given, along with the starting position of the repeat with respect to both the contig id and the specific GenBank accession id. If the repeat is found in a gene, the name of the gene and the

respective regions are shown. In the event that a repeat lies in an intergenic region, the nearest downstream gene and the distance between the repeat and the gene is given. We have also presented the association of SSRs with sequence-tagged sites (STS) in terms of distance between the SSR and the known STS marker. The details of each repeat type are included in the additional data files.

Mononucleotide repeats

All human chromosomes showed a more-or-less similar density of monomer repeats (Figure 2a). Compared to other chromosomes, exonic regions of chromosomes 7 and 16 showed maximum density - 6,063 bp/Mb and 5,928 bp/Mb, respectively - while the exonic region of Y showed the lowest (786 bp/Mb) density of monomer repeats. With the exception of chromosome 19, monomer repeat density in the non-coding region was comparable in all other chromosomes. Interestingly, however, the intronic density was slightly but consistently higher than that of intergenic regions of all chromosomes (Figure 2a). Chromosome 19 showed the highest overall monomer density. Among the two types of monomer repeats, poly(A) (or poly(T)) was far more abundant than poly(C) (or G) in all chromosomes (Figure 3). Chromosome 19 had the maximum repeat density of A (or T)

(7,429 bp/Mb) followed by chromosomes 17, 22 and 16. Chromosome 16 contained the maximum density (96 bp/Mb) of C (or G) repeats (Figure 3).

Dinucleotide repeats

Analysis of the repeats AC, AG, AT and CG showed two interesting features: first, exonic regions showed lower overall density of these repeats compared to non-coding regions, and, second, within the non-coding region, as in the case of monomer repeats, introns had a higher dimer repeat density than the intergenic region (Figure 2b). The densities of dimer repeats in both intronic and intergenic regions were comparable and more-or-less uniform across the chromosomes. Among the dimer repeats, AC and AT were predominant, whereas CG repeats were rarest (Figure 4). The density of AT repeats showed the greatest variation from one chromosome to another. For instance, chromosomes 3, 4, 11, 12, 13, X and Y had an AT density comparable to that of AC, whereas in all other chromosomes it was found to be much less than AC. The density of AG repeats was remarkably uniform across all chromosomes except for 19 and X, which had marginally greater numbers of this repeat. For details of individual dimer repeats in exonic, intronic and intergenic regions, see additional data files.

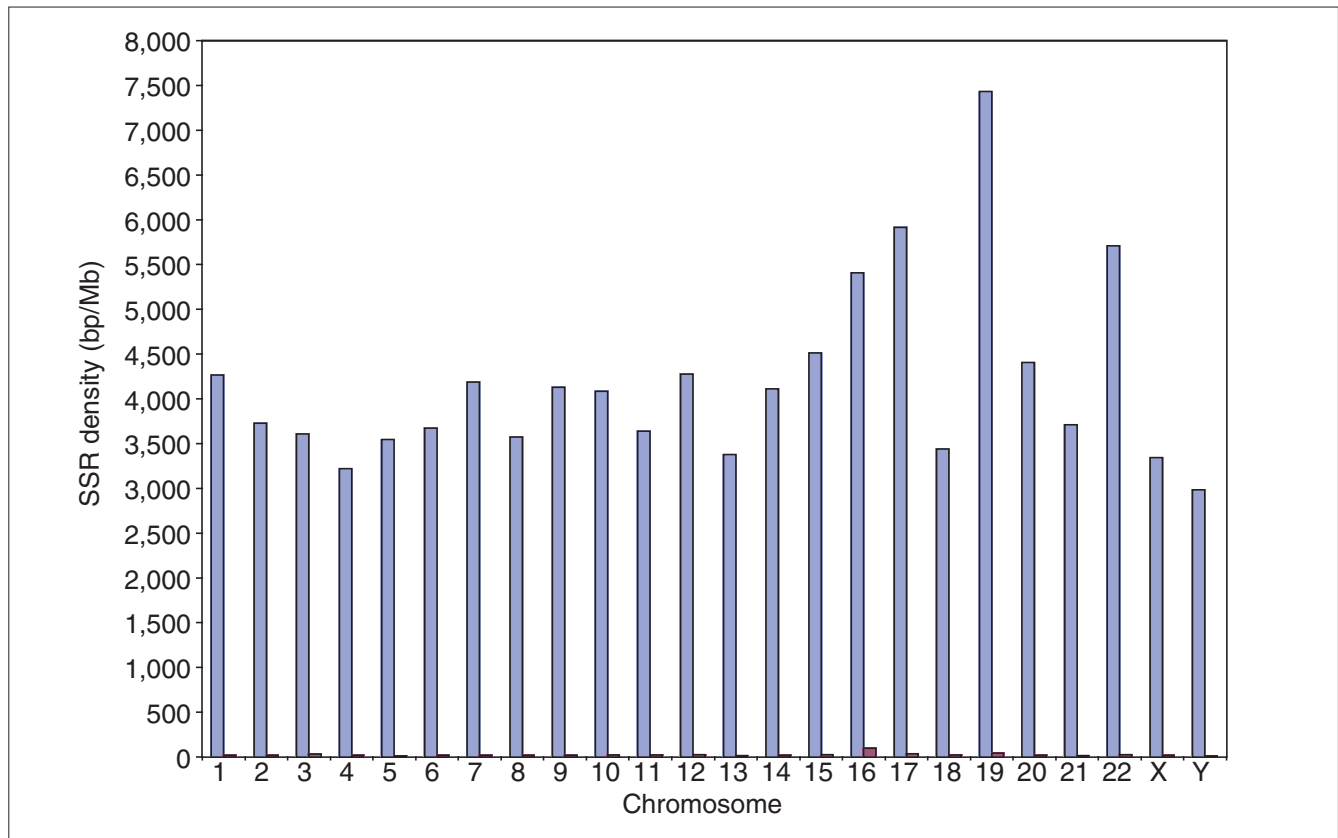
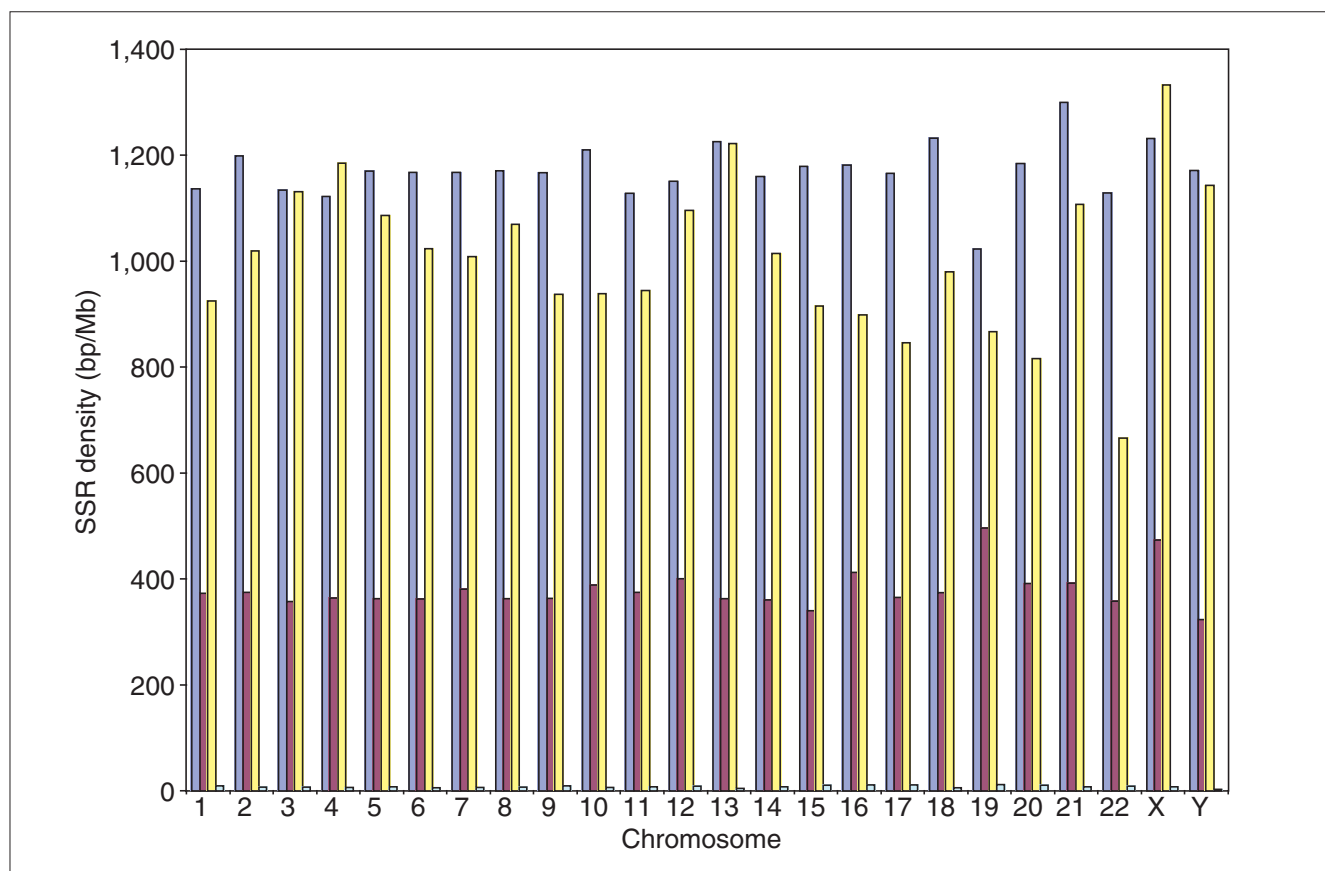


Figure 3
Density of each monomer repeat across the entire human chromosome set. A, blue; C, red.

**Figure 4**

Density of each dimer repeat across the entire human chromosome set. AC, blue; AG, red; AT, yellow; CG, turquoise.

Trinucleotide repeats

Analysis of all trimer repeats (AAC, AAG, AAT, ACC, ACG, ACT, AGC, AGG, ATC, CCG) revealed a clear greater density of approximately twofold in exonic regions compared to the density in intronic and intergenic regions in all chromosomes except Y (Figure 2c). Whereas densities of trimers in non-coding regions of all chromosomes were comparable, chromosome 19 showed the maximum density in non-coding regions. Analysis of densities of different trimers revealed that repeats of AAT, AAC, AAG and AGG were most common, in that order of abundance (Figure 5). ACG, ACT and CCG repeats were found to be much rarer in the genome. Density of the specific repeat type was found to be rather uniform across all chromosomes. There were very few deviations from this pattern; for example, chromosome 19 showed a density of the AAT repeat (446 bp/Mb) which is much higher than that of other chromosomes. For details of individual trimer repeats in exons, introns and intergenic regions see additional data files.

Tetranucleotide repeats

The density of tetramer repeats in exonic regions is less than in intronic and intergenic regions in most of the chromosomes.

Most human chromosomes showed a similar exonic density pattern; however, chromosomes 7 and 22 showed a greater density of these repeats (Figure 2d). Chromosome 19 contained most tetranucleotide repeats in the non-coding region. Repeat density in the intronic regions of other chromosomes ranged between 2,085 bp/Mb (chromosome 5) to 3,172 bp/Mb (chromosome 22). Analysis of densities of each tetrameric repeat type revealed that AAAT, AAAG, AAAC and AAGG were the predominant types across the genome. The overall densities of tetrameric repeats such as AACC, AACT, AACG, AAGC, AAGT, AATC, ACCC, ACCG, ACCT, ACGC, ACGG, ACGT, AGCC, AGCG, AGCT, ATCG, ATGC, CCGG and CCCG were less. AGAT repeats, however, predominated on the Y chromosome (Figure 6). Details of individual tetramer repeats in exons, introns and intergenic regions are given in the additional data files.

Pentanucleotide repeats

In chromosomes 4, 7, 13 and 14, pentamer repeat density in the exonic regions was greater compared to that in the non-coding regions. Chromosome 7 contained the maximum exonic repeat density (921 bp/Mb). The exonic density of pentamers ranged between 362 bp/Mb in the Y chromosome

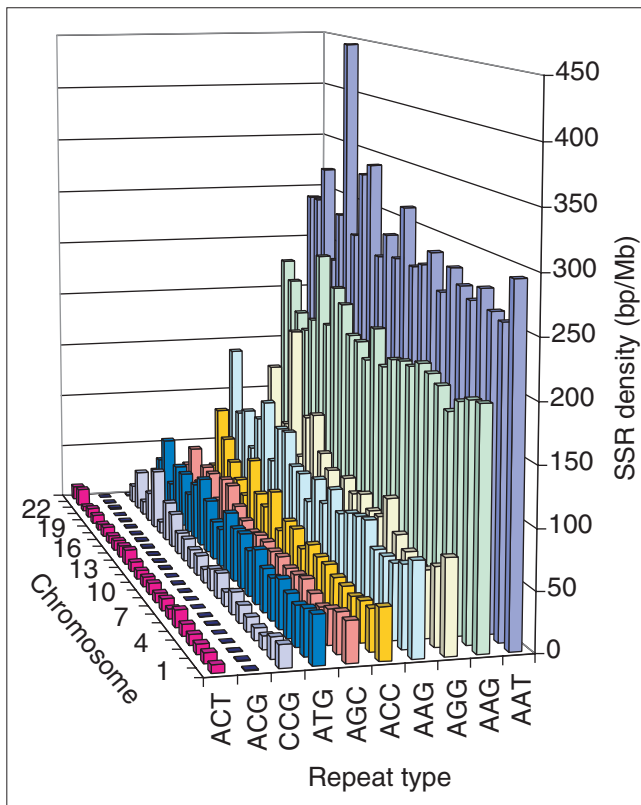


Figure 5
Density of each trimer repeat across the entire human chromosome set.

to 885 bp/Mb in chromosome 15. The intergenic densities were greater in chromosome 3, 10, 16, 17, 19 and Y (Figure 2e). An outstanding feature of pentameric repeats was noticed in the case of chromosome 19, where the intronic and intergenic density was about twofold (1,195 and 1,201 bp/Mb respectively) greater than the exonic density (633 bp/Mb). The abundance of all pentameric repeats, including the repeat number in each locus, is given in the additional data files.

Hexanucleotide repeats

Like trimer repeats, hexamer repeats are also more abundant in the exononic region, except in the Y chromosome (Figure 2f). Surprisingly, however, the density of hexameric repeats was about two- to threefold more than that of trimeric repeats in the exons. Chromosomes 7, 16 and 22 showed an increased density of exononic hexamer repeats when compared to other chromosomes. The exononic repeat density in other chromosomes, including Y, ranged from 2,176 bp to 4,628 bp/Mb. Most of the chromosomes showed remarkably uniform hexameric densities in the intronic and intergenic regions (Figure 2f). The details of densities of all the hexameric repeat and the repeat lengths of each hexameric repeat in human genome are given in the additional data files.

Discussion

SSRs contribute significantly to the human genome and are present in an abundance comparable to the coding region. Very little is known about the biological significance of this part the genome. Comprehensive analysis of SSRs is likely to be helpful in understanding their importance. Information on their abundance, coupled with the distribution patterns in the coding, as well as non-coding, regions of the genome may give us some clue to the function of SSRs in gene regulation. For example, allelic variations of HUMTHO1, a TCAT repeat, have been correlated with quantitative and qualitative changes in the binding of ZNF191 protein which, in turn, significantly influences the expression of quantitative genetic traits [14]. In our earlier analysis we found that particular repeats are preferentially associated with sex chromosomes and their frequency of occurrence in various genomes reflects an evolutionary correlation [15].

In the present study we have analyzed the occurrence and density of SSRs across the human genome. We present data on an individual chromosome basis and each chromosomal dataset has been split into exononic, intronic and intergenic regions. The overall density of SSRs in each chromosome was found to be comparable (Figure 1). The data have been analyzed taking mono-, di-, tri-, tetra-, penta- and hexamers as the six classes of repeats. Again, the density of each class of repeat is comparable across various genomic regions (Figure 2). However, different repeat motifs often show tremendous variation in density in different genomic regions, sometimes even in a chromosome-specific manner.

As is evident from Figures 3-6, within one class of repeats there may be a lot of difference in the abundance of a particular sequence repeat. In the case of mononucleotide repeats, for example, the density of poly(A) or poly(T) is ≥ 300 -fold more than that of poly(G) or poly(C). Similarly, in the case of dimeric repeats, AC and AT are the most abundant and CG is the least abundant. While some of this variation can be explained on the basis of the A/T richness and the relative ease of strand separation compared to C/G tracts [2], it cannot all be. The AC repeat, for example, is twice as abundant as the AG repeat in all chromosomes. Predominant repeats in the various classes are AAT, AAC and AAG among trimers, AAAT, AAAC, and AAAG in the case of tetramers, AAAAT and AAAAC in the case of pentamers and AAAAAT, AAAAAC, AAAAAAG and AAAAAAG among hexamers. It is possible that during SSR evolution the poly(A) stretches present in the genome might have mutated to produce the A-rich repeats. It is also possible that the abundance of repeats is influenced by their secondary structures and the effect on DNA replication. If a repeat sequence is selected during evolution for transcriptional regulation or is a target of a binding protein for one or more nuclear processes (such as chromatin organization, DNA replication, transcription, recombination), its abundance and distribution is expected to be controlled. It is

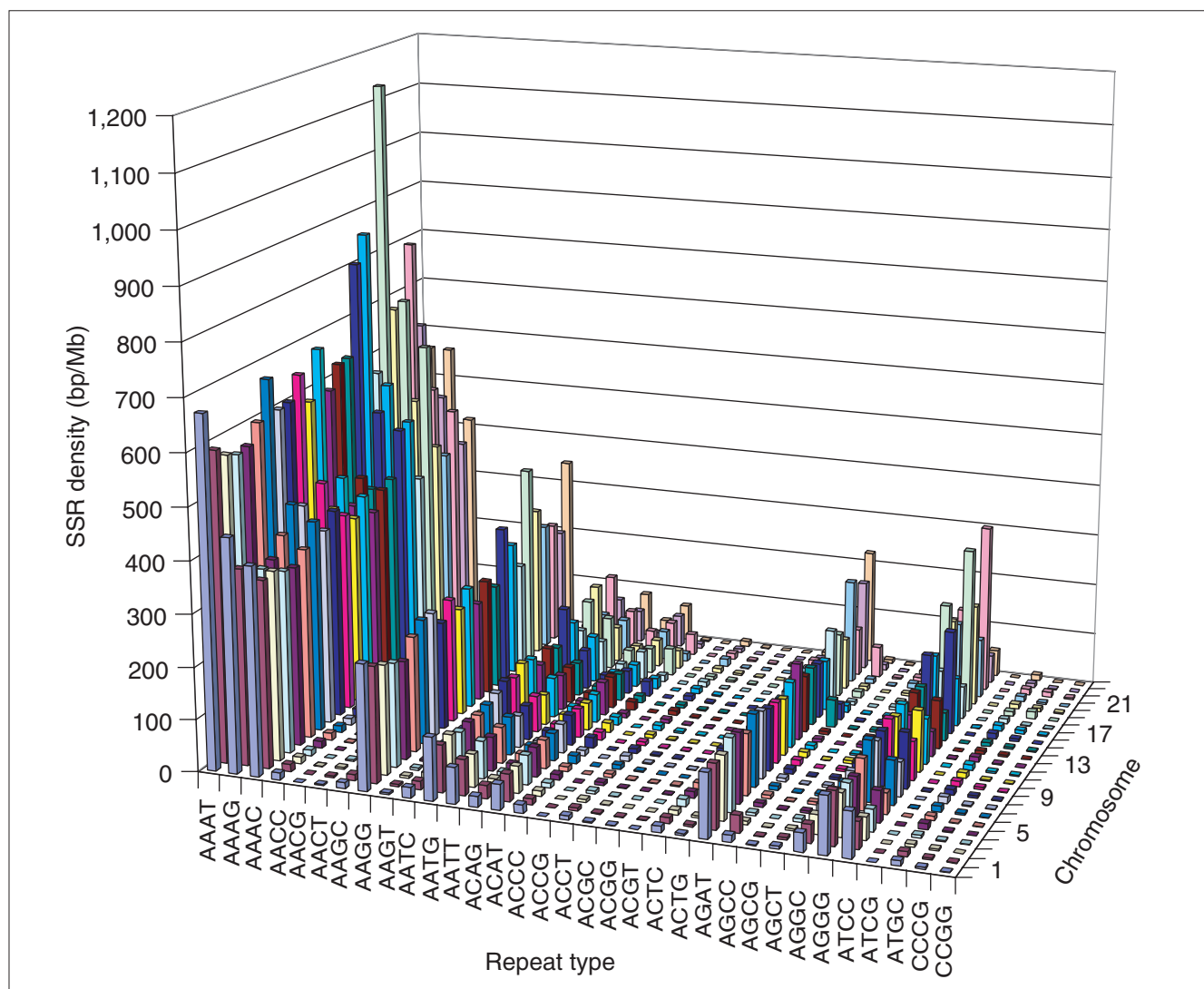


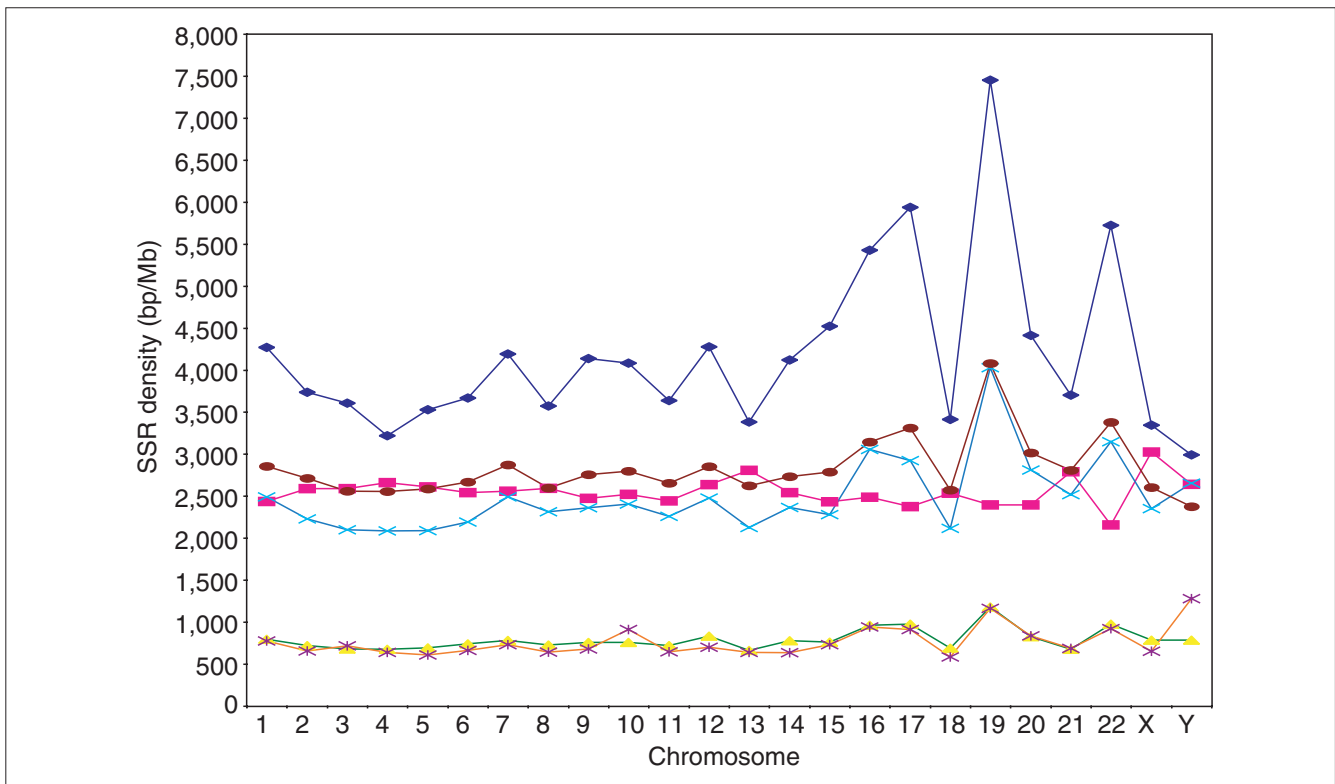
Figure 6
Density of each tetramer repeat across the entire human chromosome set.

interesting to note, in this respect, that certain repeats are very rare in the genome (see additional data files). Further studies will be required to see whether differential abundance of some repeats can be explained by any of these hypotheses. Recent studies on GT tandem repeats in human chromosome 22 revealed that they are recombination hot spots [16].

We have also compared the relative abundance of the six types of repeat over the whole chromosome set (Figure 7). Mononucleotide repeats are the most abundant class of SSR in all the chromosomes. The data showed several unexpected trends in the densities of the SSRs. The most surprising observation was that the abundance of di-, tetra- and hexamer repeats was significantly different from that of tri- and pentamer repeats. This situation, where even-number

repeats consistently fall into one group and odd-number repeats into another, was seen in all the chromosomes. Furthermore, even-number repeats (di-, tetra- and hexamers) were three times or more abundant than odd-number repeats (tri- and pentamers) (Figure 7). Among the even-number repeats, the density of tetrameric repeats was less than that of hexameric and dimeric repeats for most chromosomes. This difference in abundance might be due to positive selection of even-number repeats relative to odd-number repeats. Alternatively, there could be a more passive reason, namely that even-number repeats might be favored to accumulate and or to be maintained. Further studies will be required to test these possibilities.

When individual chromosomes are compared, chromosome 19 has several interesting distinctions. It contains the

**Figure 7**

Comparison of densities of SSRs from monomer to hexamer. Dark-blue diamonds, monomers; pink squares, dimers; yellow triangles, trimers; blue crosses, tetramers; magenta stars, pentamers; brown circles, hexamers.

highest overall density of repeats, and, whereas exonic regions are comparable in density, non-coding regions contain a high abundance of mono-, tetra- and pentamer repeats (Figures 1, 2). The other chromosome with a distinct SSR profile is Y. Although the overall abundance of repeats in Y is comparable to other chromosomes, the exonic region contains significantly fewer occurrences of all SSRs compared to other chromosomes.

An example of a repeat that is more abundant in Y than in other chromosomes is one of the pentameric repeats. Pentanucleotide repeats have a density of approximately 700 bp/Mb in all chromosomes, with few deviations (of up to twice this value). When we looked at the individual pentameric repeats, however, we came across significantly uneven occurrences in different chromosomes (see additional data files). The most striking example is AATGG. The density of this repeat varies up to 1,000-fold in different chromosomes; for example, 904 bp/Mb (chromosome 17), 738 bp/Mb (chromosome Y), 0.44 bp/Mb (chromosome 21) and 0.9 bp/Mb (chromosome X). Distribution of these repeats within a chromosome may not be uniform. We have shown previously that in the Yq centromere and in the distal end of the Yq euchromatic region there are 1,000 copies of this pentameric repeat [17].

In humans, microsatellites in the form of trinucleotide repeats can be found in genes that are associated with several neurological diseases. The association of triplet repeats with genes provides a basis for identifying genes that may be predisposed to expansion. Expansion of repeats, which is suggested to be a consequence of replication slippage, may also influence the packaging of the DNA and may have regulatory implications in some cases. Analysis of triplets has revealed that these repeats are predominantly present in exons rather than in introns and intergenic regions. A similar situation has also been found for the hexameric repeats. If one assumes that simple repeats are those that occur randomly on the chromosome irrespective of their genomic location, the occurrence of SSRs other than repeats of triplets or of other multiples of three in a coding region will completely impair the protein function by causing frameshift mutations. Earlier studies by Toth *et al.* [1] and Metzgar *et al.* [18] on tandem repeats have shown that triplets are predominantly associated with the coding regions of the genome. In coding regions, trinucleotide and hexanucleotide repeats are more frequent than mononucleotide, dinucleotide, tetranucleotide or pentanucleotide repeats.

The study of SSRs in the human genome [19,20] is just the first step towards understanding the biology of the

non-coding DNA, which constitutes more than 98% of the human genome. Similar studies will be needed for other sequenced genomes to investigate whether SSRs may also reflect the evolutionary history of different genomes. Several observations presented here suggest that individual chromosomes may be characterized by unique SSR profiles. This is also supported by the reports of chromosome-specific repeats or chromosome-specific binding proteins [21]. These observations may lead us to an understanding of the evolution and maintenance of chromosomes in general, and of particular chromosomes, for example the sex chromosomes, in particular.

The study of SSRs may help us understand numerous aspects of genome organization and function. With the availability of several genomic sequences, we have just begun to get a glimpse of the genomic organization of eukaryotes. We need to know, for example, why some repeats are abundant and others extremely rare. Is the abundance and distribution of such repeats subject to natural selection? What is the structural and functional basis of the chromosome-specific differential abundance of particular SSRs? Studies on other kinds of DNA sequences and repeats will be needed to understand the evolution, organization and function of the genome.

Materials and methods

The complete human genome sequence downloaded from the FTP site of GenBank [22] build number 29; 16 May, 2002 has been used to generate SSR data. SSRs of k-mer repeats, (where k ranges from 1 to 6, that is, monomer to hexamer repeats) were analyzed. All theoretically possible 501 SSR types [23] were analyzed for their abundance and density per Mb. The reverse complements of these repeats were also included in the analysis. We have analyzed the distribution of perfect repeats of length ≥ 12 bp. The rationale for choosing the small cutoff value was that the SSRs are often disrupted by single base substitutions.

A JAVA-based program has been developed and used to scan the entire genome to find the abundance and distribution of these repeats in coding and non-coding regions. The occurrences of repeats in exons, introns and intergenic region have been identified from the annotation of the human genome sequence in the GenBank database. The repeat density (bp/Mb) on each chromosome was calculated by dividing the total chromosome length (in Mb) by the number of base-pairs of sequence contributed by each SSR. In the case of exonic density, both coding and non-coding exons (UTRs) were included in the analysis. In the additional data files we have referred to the 5' UTR (UTR₁) as the sequence present between the transcription start point and the beginning of the start codon of the transcript. The 3' UTR (UTR₂) is the sequence between the stop codon and the last base of the transcript.

Additional data files

The details of each SSR are available as an additional data file with the online version of this paper and at our website [24].

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