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ORIGINAL ARTICLE

CLINICAL IMPACT OF PROXIMAL AUTOSOMAL IMBALANCES

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ABSTRACT

Centromere-near gain of copy number can be induced by intra- or inter-chromosomal rearrangements or by the presence of a small supernumerary marker chromosome (sSMC). Interestingly, partial trisomy to hexasomy of euchromatic material may be present in clinically healthy or affected individuals, depending on origin and size of chromosomal material involved. Here we report the known minimal sizes of all centromere-near, *i.e.,* proximal auto-somal regions in humans, which are tolerated; over 100 Mb of coding DNA are comprised in these regions. Additionally, we have summarized the typical symptoms for nine proximal autosomal regions including genes obviously sensitive to copy numbers. Overall, studying the carriers of specific chromosomal imbalances using genomics-based medicine, combined with single cell analysis can provide the genotype-phenotype correlations and can also give hints where copy-number-sensitive genes are located in the human genome.

INTRODUCTION

Autosomal Proximal Chromosome Imbalances. The finding of unbalanced chromosomal abnormalities (UBCA) was recently reviewed and summarized from a total of 200 families. The UBCA usually involve several megabases of DNA. Carriers of such UBCA are ascertained due to adverse reproductive effects or dysmorphic and/or mentally retarded offspring; the carriers themselves have an otherwise normal phenotype. Unbalanced chromosomal abnormalities have been reported for more than 50 euchromatic regions of almost all human autosomes [1,2].

Unbalanced chromosomal abnormalities leading to gain of genetic relevant material within the autosomal centromere-near region were not comprehensively followed in the above mentioned studies [1,2]. Such centro-mere-near, *i.e.,* proximal chromosomal imbalances (C-UBCA), can be induced by small supernumerary marker chromosomes (sSMCs) [3,4] and also by intrachromo-somal duplications [4]. While the latter are rare events and no reliable data on their frequency is available, sSMCs are present in 0.043% of human beings [5]. With a given population size of 7×10^9 individuals, 3×10^6 sSMC carriers are presently alive. As ~2/3 of these do not show any symptoms, $\sim 2 \times 10^6$ do not even know of their condition. Euchromatin is present in ~36.0% of those sSMC cases that do not lead to any clinical symptoms (Table 1) [6]. *N.B.*: sSMC, irrespective of origin and genetic constitution may cause fertility problems, especially in males [7]. Thus, infertility was not considered as an 'abnormal phenotype' in this study.

Even though partial trisomy is the most frequent imbalance induced by sSMC, tetra- or even hexasomy

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Table 1. Summarized here are 478 autosomal derived sSMC cases, which are characterized in detail for their size and genetic content; all of them can be found on the sSMC homepage [6]. All these cases are not associated with any clinical abnormalities. In 174, *i.e.,* 36.4%, proximal euchromatic material was present. As can be seen, the rates of cases with and without euchromatin vary from chromosome to chromosome. In general, in acrocentric derived sSMC, cases without euchromatin are in the majority, while it is the other way round in most non acrocentric derived sSMC.

Chromosomes	Cases With Euchromatin	Cases Without Euchromatin
1	4	11
2	9	2
3	10	2
4	1	0
5	8	5
6	1	1
7	1	0
8	9	2
9	18	1
10	6	1
11	2	1
12	6	2
13	1	0
14	6	53
15	35	136
16	11	8
17	2	0
18	9	2
19	5	1
20	6	6
21	8	1
22	16	69
Overall	**174**	**304**

of proximal euchromatin may be present in clinically healthy individuals [6]. Here we present the latest known proximal, centromere-near regions and their minimal molecular borders. The corresponding index cases were previously published and are summarized on the sSMC homepage [6]. This study intends to give a review on the clinical impact of proximal autosomal imbalances. A special focus is hereby laid on gain of copy numbers. For this, the following steps were necessary: ***i)*** define the pericentric regions that can be present as additional copy(ies) without causing any clinical phenotype. ***ii)*** After the definition of such copy number insensitive regions, in a second step, proximal autosomal regions including genes potentially sensitive to copy numbers can be defined. ***iii)*** Such copy number sensitive regions can be correlated with specific, typical symptoms; the latter already being possible for nine centromere-near regions in this study and there will be more in the future.

What Can be Learned From Cases With Chromosome Imbalances? It was nicely summarized back in 1993 [8] that structural autosomal imbalances may lead in 'typical cases' to syndromes with a complex of minor anomalies and/or congenital malformations. The latter 'suggests the importance of gene interaction in determining the phe-notypic picture of autosomal imbalance syndromes' [8]. Duplication-related syndromes are much more frequent than deletion-related ones, and thus, it is common sense that in general, duplications of several Mb in size are better tolerated by the human genome than deletions of the same size. This has also recently been confirmed on the level of micro-duplications and -deletions [9]. Overall, chromosomal imbalances can point towards dosage sensitive genes being responsible for specific syndromes or clinical features. A good example is the dosage sensitive peripheral myelin protein 22 (PMP22) gene in 17p11.2: a duplication of 1.4 Mb including PMP22 leads to the hereditary motor and sensory neuropathy type 1A and the reciprocal deletion to the hereditary neuropathy with liability to pressure palsies. However, also specific mutations in PMP22 itself can cause the identical syndromes [10].

Moreover, UBCA of several Mb in size have been reported, which surprisingly, do not have any clinical consequences [1,2]. At the same time, they are not pure copy number variants (CNV) such as those recently found for a cytogenetically visible amplified region in 8q21.2 [11]. In summary, there are genetically relevant regions which can be tolerated if 'amplified' as three or more copies; the reason for that is most likely that they do not comprise dosage sensitive genes. In summary, studying carriers of specific chromosomal imbalances can provide genotype-phenotype-correlations, and also give hints as to where copy-number-(in)sensitive genes are located in our genome.

Where to Find Proximal Chromosome Imbalances in Humans. Centromere-near imbalances may principally appear as deletions or duplications. However, practically no reports of proximal deletions are available in the literature. The only exceptions are offspring of carriers with an sSMC formed by the McClintock mechanism [12], *e.g.,* as reported for a child

having the karyotype 47,XY,del(2) (p12p11.1) due to a maternal cytogenetic condition (47,XX,del(2) (p12p11.1),+r(2)(::p12→p11.1::) [13]. Only eight corresponding cases are available in the literature [6] and all these patients were severely affected.

The best suited patients to study proximal duplications would be those with proximal intrachromosomal rearrangements, as direct or inverted duplications or unbalanced insertions, because these cases would be non mosaic [4]. However, such cases are scarce (summarized in Table 2). Most of these ~200 cases were only studied cytogenet-ically and no information on the molecular size of their duplicated region is available [6].

In contrast, the largest and best characterized group where to find proximal duplications are patients with sSMC [3-7,14-16]. Besides their cytogenetic characterization, more and more cases were characterized at the molecular level by array-comparative genomic hybridization (aCGH) studies [4,17,18]. However, when analyzing this group of patients one has to consider the following drawbacks: ***i)*** sSMC carriers may be mosaic with normal cell lines and/or may have different levels of mosaicism in different tissues; thus, harmful sSMC sizes may be rated as harmless [19], and ***ii)*** also harmless sSMC may be considered to be harmful if they appear together with a uniparental disomy (UPD) [20], or a mutation in a monogenic disorder gene [21]. Thus, results for regions including or excluding most likely dosage-sensitive genes, *i.e.,* C-UBCA, have to be handled carefully. Nevertheless, sSMC carriers are much more frequent and better characterized on the molecular level than intrachromosomal duplications, and are thus used here as a model system for proximal duplications.

MATERIALS AND METHODS

This study is based on the data summarized on the sSMC-homepage [6]. All raw data is freely available and can be followed down to each individual case. The data used for the present study is summarized in Tables 1 through 4.

Proximal Chromosomal Imbalances Without Clinical Consequences. The available in detail characterized sSMC cases [6] were studied by various approaches. In the majority of cases, the sSMC were characterized exclusively by molecular cytogenetics and the breakpoints are given as cytobands without molecular assessment of the exact breakpoint. In addition, there are already numerous sSMC cases characterized by well-defined locus-specific probes used in fluorescence *in situ* hybridization (FISH) and/or by aCGH [6]. In Table 3, the presently characterized C-UBCA are summarized. Overall, it could be shown that at least 96.8 Mb of the proximal chromosomal regions are tolerated as triplicates or more (Table 3). While for proximal 6q there is neither molecular nor cytogenetic hint for any dosage independent C-UBCA, in all other proximal autosomal parts at least cytogenetic evidence for C-UBCA in healthy individuals is there.

Except for proximal parts of 1q, 6p, 6q and 13q, there are molecular hints on C-UBCA for every chromosome arm, being at least between 0.07 and 10.23 Mb in size. According to cytogenetics, no less than 16 of the 39 autosomal proximal non dosage sensitive regions (= C-UBCA) are larger than already proven by aCGH, *i.e.,* 2p, 3p, 3q, 6p, 8p, 8q, 9p, 9q, 10p, 10q, 11p, 12p, 19p, 19q, 20p and 22q (Table 3).

Table 2. The ~200 case reports of proximal intra-autosomal duplications are summarized per autosome and distinguished in clinically normal and abnormal cases [6].

Chromosomes	Clinically Normal	Clinically Abnormal
1	1	8
2	0	5
3	0	1
4	0	2
5	0	4
6	0	3
7	0	2
8	0	2
9	4	2
10	1	4
11	3	3
12	0	11
13	0	2
14	0	1
15	32	>50
16	2	16
17	0	5
18	22	3
19	0	0
20	0	4
21	0	3
22	3	3
Overall	**68**	**>134**

Table 3. All 39 proximal autosomal regions containing no copy number-sensitive genes are summarized. According to the sSMC-homepage [6], the positions and sizes of duplications are given in columns 2 and 3. Column 4 summarizes if the C-UBCA may be larger according to non molecular cytogenetic results. Additionally, in the last two columns it is indicated if the C-UBCA is based on mosaic or non mosaic sSMC cases, and if more than three copies were present in the corresponding index cases. (UCSC: University of California Santa Cruz genome browser; http://genome.ucsc.edu).

Chromosomes	Molecular Bands (UCSC hg18, 2006)	Size (Mb)	Region Expected to be Larger According to Molecular Cytogenetic Results	Mosaic	>Three Copies
1p	118.33-121.10	2.80	[+/–]	(–)	–
1q	142.40-??.??	n.a	[++]	+	–
2p	89.60-91.00	1.40	[++]	+	{+;4}
2q	95.70-101.58	5.88	[+/–]	+	{+;4}
3p	87.60-89.40	1.80	[++]	–	{+;4}
3q	93.20-96.01	2.81	[++]	+	{+;4}
4p	44.03-48.70	4.67	[+/–]	+	–
4q	52.40-62.63	10.23	[+/–]	+	–
5p	37.21-45.80	1.41	[+/–]	–	{+;4}
5q	50.50-55.27	4.77	[+/–]	+	{+;4}
6p	??.??-58.40	n.a.	[++]	+	–
6q	63.40-??.??	n.a	[+/–]	n.a.	n.a.
7p	56.45-57.40	0.95	[+/–]	+	–
7q	61.10-67.00	5.90	[+/–]	+	–
8p	42.50-43.20	0.70	[++]	+	{+;4}
8q	48.10-48.30	0.20	[++]	+	–
9p	42.96-46.70	3.74	[++]	+	{+;4}
9q	70.00-70.50	0.50	[++]	+	–
10p	34.75-38.80	4.05	[++]	(–)	–
10q	42.10-43.82	1.72	[++]	–	–
11p	50.95-51.40	0.45	[++]	–	–
11q	56.40-60.23	3.83	[+/–]	+	{+;4}
12p	28.47-33.20	4.73	[++]	+	–
12q	36.50-39.90	3.40	[+/–]	+	–
13q	18.40-??.??	n.a.	[++]	–	+;4
14q	19.10-19.88	0.78	[+/–]	–	+;4
15q	18.40-21.05	2.65	[+/–]	–	+;4 +;6
16p	28.86-34.40	5.54	[+/–]	–	–
16q	45.50-46.02	0.52	[+/–]	–	{+;4}
17p	18.68-22.10	3.42	[+/–]	+	–
17q	23.20-23.32	0.12	[+/–]	+	–
18p	12.80-15.40	2.60	[+/–]	(–)	{+;4}
18q	17.30-18.12	0.82	[+/–]	+	–
19p	22.98-26.70	3.72	[++]	+	{+;4}
19q	30.20-36.90	6.70	[++]	+	–
20p	24.96-25.70	0.74	[++]	+	{+;4}
20q	28.40-29.93	1.53	[+/–]	+	{+;4} {+;6}
21q	13.20-14.85	1.65	[+/–]	–	{+;4}
22q	16.30-16.37	0.07	[++]	–	–

+/–: no larger C-UBCA expected; (–): in part mosaic index cases; n.a.: not available; ++: larger according to molecular cytogenetic results; {}: mosaic; –: no mosaic; +: mosaic; +;4: four copies; +;6: six copies.

Table 4. Clinical consequences of larger proximal autosomal imbalances for nine corresponding regions are summarized [6]. Common specific clinical symptoms were observed in crucial parts of the 5-12 cases, each; for 4q only two cases were available, both showing overgrowth. Unspecific symptoms such as mental retardation developmental delay or dysmorphic face were neglected but normally also present in these cases. For 22q cases with cat eye syndrome were excluded.

Symptom – Chromosome Region	1p	1q	4q	5q	7q	10p	17p	18q	22q
Autism	+	–	–	–	–	–	–	–	–
Finger/toe/foot malformations	+	–	–	–	+	–	–	–	–
Growth retardation	–	+	–	–	–	+	–	–	–
Heart defects	–	+	–	–	–	–	–	–	–
Hernia	–	–	–	+	–	–	+	–	–
Hypotonia	+	–	–	+	–	–	–	–	+
Macrocephaly	–	–	–	+	–	–	–	–	–
Overgrowth	–	–	+	–	+	–	–	–	–
Seizures	–	–	–	–	–	–	–	–	–
Urethral problems	–	–	–	–	–	–	–	+	+

Twenty-four of the 38 informative proximal autosomal regions are based on mosaic sSMC cases. Thus, the data summarized in Table 3 is still to be considered as preliminary in those cases, even though in >99.0% of sSMC cases, mosaicism detected in peripheral blood plays a minor role for the clinical outcome [22]. Mosaicism may play a role for the phenotype if its rates are variant in different tissues of the body [23]. The C-UBCA regions 1p, 3p, 5p, 10p, 10q, 11p, 13q, 14q, 15q, 16p, 16q, 18p, 21q and 22q were reported in non mosaic cases. The remaining regions await such proof.

Another issue to be reflected is the copy number of a C-UBCA tolerated by the human genome. At least, for 15 C-UBCA low mosaics (maximum 20.0%) of cells having four (or in one case of 20q up to six) copies of the corresponding regions are tolerated. The C-UBCA of chromosomes 13q, 14q and 15q can be present in four copies in normal carriers in 100.0% of the studied cells. For 15q, even six copies are possible (Table 3).

Autosomal Proximal Imbalances Leading to Clinical Consequences. In case an sSMC or an intrachromo-somal duplication is larger than the critical region for harmless sSMC, as summarized Table 3, a variety of clinical problems can be the consequence for the sSMC carrier. Besides well-known syndromes such as isochromosome-12p (Pallister-Killian syndrome) [24], -15q [25], -18p [26] or -22q (cat-eye-syndrome) [27], a variety of symptoms can be associated with an sSMC-induced imbalance [3,6]. In most cases the correlated symptoms are rather non specific. However, first potentially specific symptom combinations for nine corresponding imbalances are summarized in Table 4. In future, it should be possible for at least some of these proximal autosomal imbalances to define new, possibly even clinically recognizable, syndromes [3].

CONCLUSIONS

The sSMC are a long time underestimated source for the understanding of proximal chromosomal imbalances in humans. New information on regions of the human genome, possibly inert to copy number changes, can be acquired from this group of patients. Moreover, effects such as heterochromatization [3] or feedback-loops in gene regulation [28] might also be considered for the understanding of the effects of such imbalances. Comprehensive studies of more aberrant cases will also lead to new genotype-phenotype correlations and to the possibility of a clinical sub-differentiation of more sSMC cases. All these goals can only be achieved by a sophisticated balance of single cell analysis (such as in mosaic cases) and genomics-based medicine (such as for array-based approaches).

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