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Mitotic Recombination of Chromosome Arm 17q as a Cause of Loss of Heterozygosity of *NF1* in Neurofibromatosis type 1-associated Glomus Tumors

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Abstract

Neurofibromatosis type 1 (NF1) is a common, autosomal dominant, tumor-predisposition syndrome that arises secondary to mutations in *NF1*. Glomus tumors are painful benign tumors that originate from the glomus body in the fingers and toes due to biallelic inactivation of *NF1*. We karyotyped cultures from four previously reported and one new glomus tumor and hybridized tumor (and matching germline) DNA on Illumina HumanOmni1-Quad SNP arrays ($\sim 1 \times 10^6$ SNPs). Two tumors displayed evidence of copy-neutral loss of heterozygosity of chromosome arm 17q not observed in the germline sample, consistent with a mitotic recombination event. One of these two tumors, NF1-G12, featured extreme polyploidy (near-tetraploidy, near-hexaploidy, or near-septaploidy) across all chromosomes. In the remaining four tumors, there were few cytogenetic abnormalities observed, and copy-number analysis was consistent with diploidy in all chromosomes. This is the first study of glomus tumors cytogenetics, to our knowledge, and the first to report biallelic inactivation of *NF1* secondary to mitotic recombination of chromosome arm 17q in multiple NF1-associated glomus tumors. We have observed mitotic recombination in 22% of molecularly-characterized NF1-associated glomus tumors, suggesting that it is a not uncommon mechanism in the reduction to homozygosity of the *NF1* germline mutation in these tumors. In tumor NF1-G12, we hypothesize that mitotic recombination also “unmasked” (reduced to

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homozygosity) a hypomorphic germline allele in a gene on chromosome arm 17q associated with chromosomal instability, resulting in the extreme polyploidy.

Introduction

Neurofibromatosis type 1 (NF1) is a common, autosomal dominant, tumor-predisposition syndrome that arises secondary to mutations in the tumor suppressor gene *NF1* (Wallace et al., 1990). The disorder is associated with multiple types of benign and malignant tumors that arise from biallelic inactivation of *NF1*, due to loss of heterozygosity or somatic mutation (Brems et al., 2009a). Glomus tumors are painful benign tumors that originate from the glomus body, a thermoregulatory shunt concentrated in the fingers and toes. Glomus tumors present with a triad of hypersensitivity to cold, severe paroxysmal pain and focal pain at the fingertip, and are treated by surgical excision. There can be a delay in diagnosis of many years, at a cost of significant pain and morbidity. The key to diagnosis is clinical suspicion, although magnetic resonance imaging may be useful. Glomus tumors occur sporadically, typically in middle-aged females, who almost always have a single tumor (Stewart et al., 2010). We recently reported genetic, functional and clinical evidence that glomus tumors are part of the tumor spectrum of NF1 (Brems et al., 2009b). When associated with NF1, the glomus tumors are often multi-focal and can recur. The fingers are typically affected, although the tumors can occur in toes as well. Although more common in adults, children can also be affected. Glomus tumors in NF1 are more common than previously recognized and may affect up to 5% of the adult NF1 population. We have recommended that adults with NF1 are screened for glomus tumors by asking the simple question, “Do the tips of your fingers ever hurt, especially when cold or bumped?” (Stewart et al., 2010)

To our knowledge, no cytogenetic analysis has been described in glomus tumors, either sporadic or NF1-associated. In this report, we describe the cytogenetics and high-resolution SNP array-based copy-number analysis of five NF1-associated glomus tumor cultures. Clinical details, *NF1* mutation data and oligoarray analysis (but not karyotyping) have been previously reported for three of these tumors (NF1-G1, NF1-G3, NF1-G8) (Brems et al., 2009a); clinical data only has been reported on NF1-G12 (“Leu-8” in (Stewart et al., 2010)). We report the *NF1* mutation on NF1-G12 and describe the *NF1* mutation in a fifth, previously unreported tumor (NF1-G13)

Materials and Methods

Glomus Tumor Source, Culture, Pathology and Genotyping

Glomus cell culture from five pathologically-confirmed glomus tumors from five individuals with NF1 was established by treating tumors overnight with collagenase (160 units/mL) and dispase (0.8 units/mL) at 37°C. Glomus cells were grown to confluency in DMEM/F12 + 10% fetal bovine serum + penicillin + streptomycin and harvested. Clinical details, germline and somatic mutations in *NF1* for tumors NF1-G1, NF1-G3 and NF1-G8 have been previously reported (Brems et al., 2009b); for these tumors, germline DNA and DNA from at least one passage were available. The clinical but not molecular details of tumor NF1-G12 were previously reported (“Leu-8” in Table 2 and Figure 3D of Stewart et al., 2010). Germline and somatic mutations in *NF1* in tumor NF1-G12 were analyzed as previously reported (Messiaen et al., 2000). For tumor NF1-G12, germline DNA as well as DNA from passages 5 and 8 and from the primary tumor itself (before culture) was available. Tumor NF1-G13 was surgically excised from a 40-year-old man with NF1 and an eight-year history of severe, progressive right 5th digit pain. Magnetic resonance imaging of the digit showed a 4 × 12 mm lesion consistent with a glomus tumor in the lateral right 5th

digit. Germline *NF1* mutation detection on NF1-G13 was performed as previously reported (Messiaen et al., 2000).

SNP Genotyping and Determination of Ploidy, Loss of Heterozygosity and Copy-number

Table 1 lists the DNA samples from cultured glomus tumor cells, uncultured (frozen) tumor and germline (peripheral white blood cells) hybridized to HumanOmni1-Quad SNP arrays ($\sim 1 \times 10^6$ SNPs; Illumina, San Diego, CA) according to the manufacturer's instructions. Allele-specific copy-number changes, ploidy and loss of heterozygosity (LOH) were determined on DNA from primary tumor, matching cell cultures and peripheral white blood cells using ASCAT (version 2.0) (Van Loo et al., 2010). Borders of permissible tumor ploidy were set between 1.6n and 6n to allow for the extreme polyploidy of the NF1-G12 tumor, except for cases NF1-G12_P5 (ploidy range set from 3n to 6n, for consistency with other NF1-G12 passages) and NF1-G13_tumor (ploidy range set from 1.6n to 3n, for consistency with the cultured NF1-G13 tumor). Copy-number analysis was performed using GenomeStudio v.20011.1 (Illumina Inc., San Diego, CA), Partek Genomic Suite 6.5 (Partek Inc., St. Louis, MO) and Nexus 5.0 (BioDiscovery Inc., El Segundo, CA) software, all per the manufacturer's instructions. The Partek analysis included selection of "Copy number analysis," followed by "Detecting amplifications and deletions," "Finding regions in multiple samples," and "Finding overlapping genes." "Genomic segmentation" was chosen as the partitioning algorithm (default parameters). The analysis in Nexus was performed using the default settings as well. For this analysis, we analyzed six tumor-germline pairs. Four pairs (NF1-G1, NF1-G3, NF1-G8 and NF1-G13) were hybridized to HumanOmni1-Quad arrays, as noted above. Lower resolution oligonucleotide array-CGH has been reported previously on NF1-G1, NF1-G3 and NF1-G8; no copy-number changes were observed (Brems et al., 2009b). The remaining two glomus tumor samples (NF1-G10_GT1 and NF1-G10_GT3) arose in two different fingers from the same individual with NF1 and were hybridized to Illumina HumanHap550v.3 SNP-arrays ($\sim 5 \times 10^5$ SNPs), as previously reported (Brems et al., 2009b). Note that for NF1-G1, NF1-G3 and NF1-G8 tumors DNA was isolated from early passage cell cultures, whereas for NF1-G13 and NF1-G10_GT1 and NF1-G10_GT3 tumors DNA was isolated from primary tumor tissues. For the NF1-G13 tumor, DNA was available from the primary tumor and matching low-passage cell culture. The raw SNP genotype data from the HumanOmni and HumanHap550 chips were analyzed separately in GenomeStudio and then combined in either Partek or Nexus applications. Manifest files (.bpm) for both types of chips were based on the NCBI36/hg18 human genome build. We searched for copy-number changes in at least three of the six tumors using either Partek or Nexus software, and then sought significant differences identified by both methods. We excluded the NF1-G12 tumor-germline pair from copy-number analysis, given the extreme polyploidy of the tumor genome.

Karyotyping and Clustering Analysis

Cytogenetic analysis and chromosome counts were performed with DAPI banding and spectral karyotyping (SKY, Applied Spectral Imaging Inc, Carlsbad, CA) on metaphase spreads from all five glomus tumors cell cultures. Higher passage cell culture was used in the karyotyping experiments: NF1-G1 (passage 11), NF1-G3 (passage 13), NF1-G8 (passage 14), NF1-G12 (passage 9) and NF1-G13 (passage 3). Metaphase slide preparations were made after mitotic arrest with colcemid (0.015 $\mu\text{g/mL}$, 2–4 hours) (GIBCO, Gaithersburg, MD), hypotonic treatment (0.075 mol/L KCl, 20 minutes, 37°C), and fixation with methanol–acetic acid (3:1). Mosaic counts were performed whenever possible. Due to the high level of heterogeneity of karyotypes of NF1-G12, we performed a clustering analysis of individual karyotypes in order to identify a predominant class. Clustering was performed in BRB-Array Tools microarray analysis software, v3.8.0 (Biometric Research Branch, Division of Cancer Treatment and Diagnosis, NCI/NIH, Bethesda, MD). Counts for

each individual chromosome in 13 metaphases were used as an input. Clustering was performed using centered correlation and average linkage per the developer's instructions.

Results

NF1-associated Glomus Tumors are Pathologically Similar to Sporadic Glomus Tumors

In general, NF1-associated glomus tumors share many histologic similarities to sporadic ones: namely the cuboidal cells surrounding blood vessels, smooth muscle actin (SMA) positivity, and benign appearing rounded nuclei with moderate to abundant cytoplasm. Both tumors NF1-G12 and NF1-G13 were large and arose secondary to mitotic recombination of chromosome arm 17q; in addition, tumor NF1-G12 was also visible as a mass at the base of the nail bed. Tumor NF1-G12 was a fibrous fragment consisting of monotonous population of small pericyte-like cells with regular, round nuclei and no atypical nuclei; the features were characteristic of a glomus tumor. Immunohistochemistry showed diffuse expression of alpha-SMA in the pericyte cells, consistent with glomus tumor; the endothelial cells of the blood vessels expressed CD31. Tumor NF1-G13 was readily recognizable as a glomus tumor but was composed of mostly larger epithelioid cells with abundant pale eosinophilic cytoplasm consistent with epithelioid glomus tumors, or oncocytic changes in a glomus tumor (Slater et al., 1987; Shin et al., 1990) (Supplementary Figure 1).

Mitotic Recombination of Chromosome Arm 17q is the mechanism of biallelic inactivation of NF1 in glomus tumor NF1-G12 and NF1-G13

Table 1 summarizes the germline and somatic mutations in *NF1* for the five samples. In tumors NF1-G12 and NF1-G13, there is evidence of copy-neutral loss of heterozygosity of chromosome arm 17q not observed in the germline sample (Figure 1), consistent with a mitotic recombination event. This was likely an early or initiating event since, unlike other copy-number abnormalities, there is no diminution of the abnormality with increased passage number. Since chromosomal band 17q11.2 harbors *NF1*, mitotic recombination is the likely explanation of biallelic inactivation of *NF1* in these tumors. No mutation in *NF1* was identified in tumor DNA from NF1-G12, other than the known germline mutation.

Karyotyping and Copy-number Analysis of NF1-G12 Reveals Extensive Polyploidy

There were few cytogenetic abnormalities observed in NF1-G1, NF1-G3, NF1-G8 or NF1-G13 (Table 1); ASCAT analysis was consistent with disomy for all chromosomes (Table 2). Glomus tumor NF1-G12 featured extensive polysomy across all chromosomes (Table 2, Supplementary Table 1 and Figure 2), but no re-arrangements or abnormalities in morphology, as determined by SKY (Supplementary Figure 2). We observed intra-tumoral heterogeneity in chromosome copy number in tumor NF1-G12. First, no two identical karyotypes were observed (Supplementary Table 1). Second, there was little similarity across clones in chromosome count, and the highest pair-wise correlation was ~0.5 (Figure 3). Third, in NF1-G12, we observed three different groups of chromosomes: near-tetraploidy (chromosomes 1, 7, 10, 11, 14, 15, 16, 22, X), near-hexaploidy (chromosomes 2, 3, 4, 5, 6, 8, 9, 21) and near-septaploidy (chromosomes 12, 13, 17, 18, 19, 20) (Table 2). The variety of abnormalities was most prominent in the primary tumor and decreased in passages 5 and 8 (Table 2 and Supplementary Figure 3 D, E, F). The decrease in heterogeneity of aberrations correlated with increased passage number and increased normal cell admixture (as determined by ASCAT), likely reflecting the growth of normal cells in culture (Supplementary Figure 3). The karyotyping was performed on cells from passage 9 (9.4% aneuploid), and thus under-estimates the true percentage of abnormal chromosomes in the original tumor, as estimated by ASCAT from frozen tumor DNA (Table 2).

Copy-number Analyses

Supplementary Tables 2, 3 and 4 summarize the copy-number analyses of the six glomus tumor-germline pairs in Partek and Nexus, respectively. Genes (or loci) with significant differences (nominal P value < 0.05 , with no multiple testing correction) in copy-number in at least 3 out of 6 tumors are listed in the tables. Specifically, two genes (*WDR17* and *C16orf11*) and one microRNA (*MIR1267*) were deleted in at least three of the six tumors studied using two different methods (Partek and Nexus). The Partek analysis identified 24 such genes, containing only deletions, while Nexus identified ten genes with copy-number differences: three with amplifications and seven with deletions. The median sizes of aberrant regions were similar in the two analyses: 1.9 and 1.5 kilobases in Partek and Nexus, respectively. There were two common genes in the two lists (*WDR17* and *C16orf11*) and one microRNA (*MIR1267*). All three genes carried deletions in tumor tissue but were normal in germline samples.

Discussion

This is the first study, to our knowledge, of the cytogenetics of glomus tumors, and the first to report biallelic inactivation of *NF1* secondary to mitotic recombination of chromosome arm 17q in multiple NF1-associated glomus tumors. Three of the five studied tumors harbored discrete pathogenic germline and somatic mutations in *NF1*. Two of the five tumors (NF1-G12 and NF1-G13) had evidence of mitotic recombination of chromosome arm 17q; since the gene *NF1* resides in chromosome band 17q11.2, mitotic recombination causes loss of heterozygosity and is thus the initiating event in the tumorigenesis of the glomus tumor. Four of the five glomus tumors studied were diploid with little evidence of aneuploidy or structural rearrangement by mosaic-count cytogenetic and SNP copy-number analysis. In only one of the four diploid tumors (NF1-G13) could DNA from the original frozen tumor be directly studied (the other three were cultured), and thus we may have underestimated the frequency of polyploidy. One tumor (NF1-G12) was polyploid across all chromosomes; this polyploidy was not a tissue-culture artifact and was also observed by analysis of allele-specific copy-number of DNA from the primary tumor. We identified two genes (*WDR17* and *C16orf11*) and one microRNA (*MIR1267*) with copy-number changes (all deletions) in at least three of the six tumors studied using two different methods (Partek and Nexus) (Supplementary Tables 2, 3 and 4). We are hesitant to draw many conclusions from such a small sample set. MicroRNAs are short non-coding RNAs involved in post-transcriptional regulation of gene expression; *MIR1267*, located in the same locus but on the opposite strand of *WDR17*, has unknown function. Similarly, *C16orf11* encodes a poorly characterized hypothetical protein. In a large sequencing study of medulloblastoma, a single missense mutation in *C16orf11* was identified in one sample (Parsons et al., 2008). Lastly, *WDR17* is expressed in retina and testis and is a candidate gene for autosomal recessive retinitis pigmentosa (Stohr et al., 2002; Roni et al., 2007; Geisert et al., 2009). In the COSMIC database, missense and synonymous mutations in *WDR17* have been occasionally reported in medulloblastoma, ovary carcinoma and malignant melanoma (Wellcome Trust Sanger Institute COSMIC database, accessed September 15, 2011).

Mitotic recombination of 17q is a common, even predominant, mechanism for LOH of *NF1* in NF1-associated tumors. Mitotic recombination has been reported in dermal neurofibromas (62%) (Garcia-Linares et al., 2011), spinal neurofibromas (75%) (Upadhyaya et al., 2009), plexiform neurofibromas (46%) (Steinmann et al., 2009), NF1-associated juvenile myelomonocytic leukemia (47%) (Steinmann et al., 2010) and gastrointestinal stromal tumors (Stewart et al., 2007). No evidence of correlation between the presence of mitotic recombination of chromosome arm 17q in NF1-associated tumors and tumor phenotype (e.g., size, growth rate or histology) has been reported. We note that the two glomus tumors

with mitotic recombination were both unusually large and NF1-G13 harbored some unusual histologic features.

Atypically, the polyploid tumor (NF1-G12) was readily apparent on visual inspection at the base of the nail. Mitotic recombination is rare, although it has been reported as an important mechanism in other genetic disorders including ichthyosis with confetti (Choate et al., 2010). In previous work (Brems et al., 2009b), we described biallelic inactivation in seven glomus tumors, all secondary to discrete mutations or LOH in *NF1*, and none secondary to mitotic recombination. Glomus tumors are rare and thus caution needs to be exercised in drawing conclusions from small sample sets. However, this report increases the number of molecularly characterized glomus tumors (NF1-GT12 and NF1-GT13) to nine, of which two (22%) from two separate populations (North America and Belgium) arose secondary to mitotic recombination, suggesting that it may be a not-infrequent mechanism in the reduction to homozygosity of the *NF1* germline mutation in these tumors.

There are numerous reported examples of NF1-associated tumors, typically malignant peripheral nerve sheath tumors, neurofibromas and leukemias, with high ploidy, although most have very complex karyotypes (Mertens et al., 2000; Wallace et al., 2000; Kobayashi et al., 2006; Liu et al., 2010; Mitelman et al., 2011). In NF1-G12 we observed high ploidy in multiple karyotypes in late-passage glomus cells but no other apparent chromosomal rearrangement other than the mitotic recombination of chromosome arm 17q (Supplementary Figure 2). This is consistent with allele-specific copy-number determination by ASCAT on DNA from the frozen (and therefore never cultured) tumor that showed near tetra-, hexa- and septaploidy in the majority of chromosomes. The diversity of observed ploidy in NF1-G12 suggests intra-tumoral heterogeneity and extensive sub-clonal structure and was not observed in the other glomus tumors.

Cell culture of tumors can distort the proportions of normal and non-normal cells; paradoxically the most abnormal, genomically deranged cells *in vivo* often do not grow well *in vitro* and can be overgrown by stromal cells (Buehring and Williams 1976; Gazdar et al., 2010). This can be seen with the increasing passage number of tumor NF1-G12; the magnitude of copy-number abnormalities decreases from the non-cultured primary tumor with passage number (Table 2 and Supplementary Figure 3 D, E, F). This is likely secondary to the growth of normal cells in culture. Thus it is possible that the cultures for NF1-G1, NF1-G3, NF1-G8 and NF1-G12 do not reflect the copy-number abnormalities present in the original tumor. Since the karyotyping was performed on higher passage tumor cell culture, the number of cytogenetic abnormalities present in the original tumor may be underestimated.

Maintenance of chromosomal stability is important in cellular homeostasis, and numerous chromosomal instability (CIN) genes have been identified (Chandhok and Pellman, 2009), including multiple candidates on chromosome arm 17q (e.g., *MYC*, *ERBB2*, *NME1*, *MPO* and *MECOM*). High ploidy is not uncommon in hematologic malignancies (Pan et al., 2009), but is less common in solid tumors (Chow and Poon, 2010) and may be either a cause or a consequence of tumorigenesis (Holland and Cleveland, 2009). More broadly, it remains controversial whether aneuploidy in general is a cause or a consequence of tumorigenesis (Chandhok and Pellman, 2009). The mitotic recombination of chromosome arm 17q was likely the initiating event in the tumor since it resulted in reduction to homozygosity of the mutated germline *NF1* allele. Abnormalities of chromosome 17 have been implicated in other high-ploidy solid tumors (Olaharski et al., 2006; Bettio et al., 2008). Further, isochromosome 17q is one of the most frequent nonrandom genomic alterations in neuroectodermal tumor, medulloblastoma, chronic myeloid leukemia, acute myeloid leukemia and myelodysplastic syndrome (Carvalho and Lupski, 2008). Although mitotic

recombination of chromosome arm 17q is common in NF1-associated tumors, high ploidy in the absence of other cytogenetic rearrangements is not, especially in benign tumors. We observed mitotic recombination in chromosome arm 17q in our tumor NF1-G13, but no polyploidy. Thus, in NF1-G12, we hypothesize that mitotic recombination also “unmasked” (reduced to homozygosity) a hypomorphic germline allele in a gene on chromosome arm 17q associated with chromosomal instability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Evidence of mitotic recombination in glomus tumors NF1-G12 and NF1-G13. A. B-allele frequency and Log R ratio plots of SNP array data of chromosome 17 (only) showing copy-neutral loss of heterozygosity of chromosome arm 17q likely secondary to mitotic recombination (NF1-G12 shown; NF1-G13 similar). B. ASCAT profile of copy-number across all chromosomes in tumor NF1-G12. X-axis = chromosome number; Y-axis = copy-number. Purple = total copy-number; Blue = copy-number of the minor allele. Note the copy-number of the minor allele for chromosome arm 17q is zero, consistent with mitotic recombination.

Figure 2.
Karyotype (124, XX) of cell 612-11 from tumor NF1-G12 (see Supplementary Table 1).

Dendrogram for clustering experiments, using centered correlation and average linkage.

Figure 3.

Clustering analysis of karyotyped metaphases in culture of glomus tumor G12. Names of individual metaphases are shown on the bottom; total number of chromosomes is shown in parenthesis. Correlation distance is shown on the left.

Table 1

Summary of *NF1* germline and somatic mutation genotyping (from Brems *et al.*, 2009 for NF1-G1, NF1-G3 and NF1-G8) and cytogenetic analysis. The somatic mutations in NF1-G3 were detected in 2 different tumors. Clinical details but not *NF1* mutations on NF1-G12 have been previously reported (“Leu-8” in Stewart *et al.*, 2010). NF1-G13 has not been previously reported.

Name of Glomus Cell Line	Mutation analysis		Cytogenetic analysis									
	Germline <i>NF1</i> Mutation	Somatic <i>NF1</i> Mutation	Number of Metaphases Counted	Number of metaphase spreads with observed chromosome count								
NF1-G1	Partial skip exon 29	c.403delC	31	Tetraploids	46	45	44	43	42	Less than 42 (actual chromosome count)		
NF1-G3	c.2546dupG	c.5545C>A, c.5539_5546dup8	21	3	28	0	0	0	0	0		
NF1-G8	c.311T>G	c.7727C>A	74	0	19	0	1	0	0	1 (38)		
NF1-G12	c.4368-1G>C	Chromosome 17q mitotic recombination (see Figure 1)	139	0	40	9	11	2	8	1 (40) and 3 (41)		
NF1-G13	c.3113+1G>A	Chromosome 17q mitotic recombination (see Figure 1)	28	0	20	4	2	1	1	0		

See Supplementary Table 1: complex karyotype

Table 2

Copy number of all chromosomes for all samples, as determined by ASCAT. Tumors NF1-G1, NF1-G3 and NF1-G8 had DNA from early passage glomus cell cultures available (“_Early”) but not from frozen tumors. Tumors NF1-G12 and NF1-G13 had DNA from glomus cell cultures and DNA from frozen tumors. P = passage number; N/A = not available

Chr.	NF1-G1_Early	NF1-G3_Early	NF1-G8_Early	NF1-G12_P5	NF1-G12_P8	G12_Tumor	NF1-G13_P2	NF1-G13_Tumor
1	2.05	2.00	1.98	2.16	3.74	4.18	1.98	3.92
2	2.05	1.97	1.97	2.94	4.09	5.65	2.01	3.96
3	2.04	1.96	1.91	2.92	4.07	5.63	1.96	3.94
4	2.04	1.90	1.85	2.91	3.87	5.64	1.96	3.87
5	2.04	1.90	1.87	2.90	4.02	5.61	1.97	3.95
6	2.04	2.00	1.89	2.90	3.99	5.60	1.99	3.89
7	2.05	2.00	1.96	2.11	3.66	4.11	2.06	4.02
8	2.07	2.04	2.02	2.93	4.09	5.66	2.07	4.08
9	2.05	1.97	1.96	2.92	4.03	5.63	2.03	3.94
10	2.06	2.02	2.03	2.18	3.77	4.20	1.97	3.98
11	2.04	1.92	1.90	2.14	3.70	4.15	1.96	3.88
12	2.05	1.98	1.93	3.50	4.31	6.65	1.95	3.91
13	2.07	1.99	2.05	2.96	4.11	6.44	2.02	3.99
14	2.04	1.93	1.95	2.14	3.70	4.14	1.96	3.88
15	2.06	1.99	2.00	2.18	3.76	4.20	2.00	4.01
16	2.05	1.96	2.01	2.23	3.76	4.30	1.93	3.94
17	2.06	1.99	2.07	3.52	4.45	6.62	2.04	3.95
18	2.07	2.03	2.07	3.04	4.10	6.41	2.04	4.01
19	2.05	1.94	1.98	3.00	3.87	6.54	1.82	4.09
20	2.07	2.08	2.10	3.52	4.49	6.68	2.03	4.18
21	2.04	1.90	1.95	2.94	3.97	5.73	1.91	3.86
22	2.04	1.86	1.97	2.27	3.70	4.40	1.87	3.79
X	2.12	2.26	2.18	2.19	3.97	4.19	N/A	N/A