

A meta-analysis of single base-pair substitutions in translational termination codons ('nonstop' mutations) that cause human inherited disease

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Date received (in revised form): 2nd March 2011

Abstract

'Nonstop' mutations are single base-pair substitutions that occur within translational termination (stop) codons and which can lead to the continued and inappropriate translation of the mRNA into the 3'-untranslated region. We have performed a meta-analysis of the 119 nonstop mutations (in 87 different genes) known to cause human inherited disease, examining the sequence context of the mutated stop codons and the average distance to the next alternative in-frame stop codon downstream, in comparison with their counterparts from control (non-mutated) gene sequences. A paucity of alternative in-frame stop codons was noted in the immediate vicinity (0–49 nucleotides downstream) of the mutated stop codons as compared with their control counterparts ($p = 7.81 \times 10^{-4}$). This implies that at least some nonstop mutations with alternative stop codons in close proximity will not have come to clinical attention, possibly because they will have given rise to stable mRNAs (not subject to nonstop mRNA decay) that are translatable into proteins of near-normal length and biological function. A significant excess of downstream in-frame stop codons was, however, noted in the range 150–199 nucleotides from the mutated stop codon ($p = 8.55 \times 10^{-4}$). We speculate that recruitment of an alternative stop codon at greater distance from the mutated stop codon may trigger nonstop mRNA decay, thereby decreasing the amount of protein product and yielding a readily discernible clinical phenotype. Confirmation or otherwise of this postulate must await the emergence of a clearer understanding of the mechanism of nonstop mRNA decay in mammalian cells.

Keywords: human inherited disease, stop codon, 3'-untranslated region, nonstop mutation, nonstop mRNA decay

Introduction

There are currently in excess of 60,000 missense and nonsense mutations (in nearly 4,000 different genes) listed in the Human Gene Mutation Database (HGMD) that are known to cause, or to be associated with, human inherited disease.¹ In addition, there are 119 examples of mutations (in 87 different genes) that occur within stop codons, a category of mutation which therefore constitutes ~0.2% per

cent of codon-changing mutations.¹ Such lesions have been termed 'nonstop', 'nostop' or 'read-through' mutations on the basis that the loss of the normal translational termination (stop) codon is likely to lead to continued translation of the mRNA further downstream into the 3'-untranslated region (UTR).

Although many authors tacitly assume that the normal open reading frame will simply be extended

until the next in-frame stop codon is encountered, too few human nonstop mutations have so far been characterised to allow any general conclusions to be drawn as to their likely phenotypic consequences at either the mRNA or the protein level. In three reported cases, however (namely, those nonstop mutations in the gene encoding ribosomal protein S19 [*RPS19*], causing Diamond–Blackfan anaemia,² the *F10* gene causing factor X deficiency³ and the foxhead box E3 [*FOXE3*] gene causing anterior segment dysgenesis⁴), the levels of the mutant mRNA transcripts were found to be dramatically lower than those of their wild-type counterparts. By contrast, the mRNA level associated with a nonstop mutation in the 3- β -hydroxy- Δ -5-steroid dehydrogenase (*HSD3B2*) gene causing adrenal hyperplasia was found to be near normal, although both *HSD3B2* enzymatic activity and antigen (associated with a predicted 467 amino-acid protein, extended by 95 residues beyond the wild-type length) were found to be dramatically reduced.⁵ Similarly, in the case of a nonstop mutation in the thymidine phosphorylase (*TYMP*) gene responsible for mitochondrial neurogastrointestinal encephalomyopathy, the mRNA level was not found to be reduced, even although the thymine phosphorylase protein product it encoded was undetectable.⁶

In yeast, nonstop mRNAs generated from mRNAs lacking translational termination codons are recognised, by the protein Ski7, on ribosomes that have become stalled at the 3' ends of the mRNAs; these RNAs are then targeted for exosome-mediated degradation.^{7–9} While this process of 'nonstop mRNA decay' is fairly effective at removing nonstop mRNAs, any protein products generated by translation of residual nonstop mRNAs are degraded by the proteasome.^{10,11} Although few such studies have so far been attempted in mammalian cells, the expression level of nonstop mRNAs generally appears unaltered while ribosome stalling at the 3' end of the elongated nonstop mRNA blocks translation before the completion of synthesis of full-length polypeptides.^{12–14}

Precisely how nonstop mRNA decay impacts upon naturally occurring human nonstop mutations

is unknown but, as is clear from the five disease-associated examples mentioned above, the evidence acquired to date suggests that this may be a gene- and mutation-dependent process.¹⁵ Thus, although not uncommon, remarkably little is as yet known about the nature and consequences of this type of mutation. In this paper, we report a first meta-analysis of naturally occurring nonstop mutations causing human inherited disease. With a view to exploring the various possible factors that could impact upon the likelihood of a given nonstop mutation coming to clinical attention, we have performed an analysis of the sequence context of the mutated stop codons and the average distance to the next in-frame downstream stop codon in comparison with control (non-mutated) gene sequences.

Methods

Mutation and control datasets

A total of 119 naturally occurring nonstop mutations from 87 human genes (Supplementary Table S1) were identified from the HGMD.¹ The majority of these nonstop mutations were single examples identified in specific genes but 18 genes harboured a total of 50 examples of this type of lesion. Since the multiple inclusion of identical sequences flanking mutated stop codons would have introduced considerable bias into the subsequent analysis, only one mutation per gene was considered in the analysis of the sequence context.

A control dataset was established which comprised 1,692 genes listed in the HGMD (for which both coding and 3'-UTRs were obtainable from Ensembl [Build 37] but for which no termination codon [nonstop] mutations have so far been recorded). Data from the Transterm database (<http://uther.otago.ac.nz/Transterm.html>),¹⁶ representing a total of 29,210 stop codons associated with annotated human genes, were used as genome-wide controls.

Analysis of nonstop mutations

The relative frequency of each type of stop codon (ie TAG, TAA and TGA) in the mutated (nonstop

mutation-bearing) sequences and non-mutated wild-type control gene sequences was assessed. Stop codons harbouring single and multiple mutations were examined separately.

To detect any bias in the pattern of stop codon mutability, the mutability of the dinucleotides within a pentanucleotide spanning the stop codon and including one flanking nucleotide on either side was assessed. The number of mutations occurring in each of the 12 possible dinucleotides (note that four dinucleotides [CC, CA, CG and TC] cannot occur in conjunction with any stop codon-spanning pentanucleotide and were therefore omitted) was counted. In the HGMD control dataset, one nucleotide position within each stop codon was randomly mutated and the numbers of mutations in each possible dinucleotide were then counted. Statistical significance was determined using Fisher's exact test with a Bonferroni correction being applied to allow for multiple testing.

Since the identity of the nucleotides immediately flanking the stop codon may influence the susceptibility of the stop codon to mutation, the frequencies of each DNA base in each of the six positions upstream and downstream of the normally used stop codon were obtained for both the mutated sequences and the controls. The expected frequency E of the DNA bases at each position was calculated based on the probability of observing this nucleotide in the HGMD control sequences:

$$E_{ij} = \frac{F_{ij}N_m}{N_c}$$

where E_{ij} is the expected frequency of the base $I = \{A, C, G, T\}$ at position j , F_{ij} is the observed frequency of base i at position j in the HGMD control dataset, N_m is the total number of mutated sequences and N_c is the number of sequences in the HGMD control dataset. Under the assumption that the data follow a binomial distribution, we considered that an increase or decrease in the observed frequency of a particular nucleotide in a specified position was statistically significant if the corresponding p value was <0.01 . In addition, to investigate whether any particular stop codon (ie TGA,

TAG or TAA) was associated with any specific flanking nucleotides, we placed both the mutated and control sequences into separate datasets for each of the three stop codons and repeated the above analysis for each of the new datasets.

Determining the distance to the next downstream in-frame stop codon

The distance to the next downstream stop codon in the required reading frame is likely to determine the length of any extended protein product. For each mutated (nonstop mutation-bearing) DNA sequence and each sequence in the HGMD control dataset, we therefore determined the distance to the next in-frame stop codon downstream. Sequences in the HGMD control dataset, for which the next downstream stop codon was beyond the 3'-UTR sequence available from Ensembl, were not used in this analysis. Distances between 0 and 500 base pairs (bp) from the original stop codon were divided into 'bins', each 50 bp long, the final bin containing all sequences where the distance was greater than 500 bp. The number of sequences which fell into each bin was recorded for both the mutated sequences and the HGMD control sequences. The same procedure was repeated for those sequences with single mutations and for those sequences harbouring two or more mutations. To assess the statistical significance of our findings, we employed Fisher's exact test using a Bonferroni correction to allow for multiple testing. p values of <0.05 were considered to be statistically significant.

Using the same method as for the original stop codons, we also investigated the frequency of occurrence of specific nucleotides surrounding the next in-frame stop codon downstream. It is possible that at least a proportion of these downstream in-frame stop codons are associated with naturally occurring splice isoforms of the gene,¹⁷ and might therefore possess comparable sequence characteristics to the stop codons involved in the mutational events. The flanking sequence may also affect the likelihood of a mutation coming to clinical attention.

Results and discussion

Relative frequency of stop codon involvement in nonstop mutation

We have performed a meta-analysis of the 119 nonstop mutations (in 87 different genes) known to cause human inherited disease (Supplementary Table S1) and recorded in the HGMD.¹ HGMD is a comprehensive collection of germline mutations causing (or associated with) human inherited disease and is an invaluable source of data for meta-analyses of human gene mutations.

The termination of synthesis of every human protein is effected by one of three stop codons, TAG, TAA and TGA, listed in increasing order of usage in human genes. We posed the question as to whether one of these stop codons might be more susceptible to mutation, or alternatively might be more likely to come to clinical attention once mutated, than the others. We noted that a majority of the nonstop mutations (57 per cent) in our dataset occurred within TGA codons (Table 1). Since 49.4 per cent and 48.6 per cent of stop codons in the HGMD control gene dataset and human genome dataset, respectively, were of this type, however, this finding did not attain statistical significance (Table 1; *p* values 0.107 and 0.066, respectively).

The proportion of mutations in the other two types of stop codon was also not significantly different from the corresponding proportions in the set of HGMD control gene sequences (*p* values, 0.674 for TAA and 0.201 for TAG) and in the human genome at large (*p* values, 0.753 for TAA and 0.88 for TAG).

The above notwithstanding, we speculated whether TAA codons flanked on the 3' side by A might be hypermutable, since this would in effect constitute a short polyadenine run. It has been reported that bases adjacent to mononucleotide runs in the human genome are characterised by an increased single nucleotide polymorphism frequency.¹⁸ We therefore assessed whether the nucleotide A following the TAA stop codon might influence the mutability of this codon. In agreement with our postulate, the presence of an A adjacent to a TAA stop codon was indeed found to increase the mutability of this codon by 1.4 fold (*p* = 0.016).

Genes exhibiting an abundance of missense/nonsense mutations do not harbour a disproportionate number of nonstop mutations

As we have noted above, a total of 18 human genes are known to harbour multiple nonstop mutations. We therefore sought to determine whether this was simply due to a particularly large number of mutations having been reported from these genes. At the time this analysis was performed (October 2010), the HGMD contained mutation data from a total of 2,249 human genes, for which a total of 55,813 missense or nonsense mutations had been reported. No correlation was found, however, between the probability of finding multiple nonstop mutations in a given gene and the total number of missense and nonsense mutations reported for that gene (Pearson's correlation -0.108; *p* = 0.67). Thus, for example, the largest

Table 1. The proportion of nonstop mutations harboured by each type of stop codon in mutated gene sequences, HGMD control gene sequences and the human genome at large

Stop codon type	Proportion of stop codons harbouring nonstop mutations causing human genetic disease (%) ^a	Proportion of stop codons in HGMD control gene sequences (%) ^b	Estimated proportion (number) of stop codons in the human genome (%) ^c
TAA	26.05	28.60	27.8 (8106)
TAG	16.81	21.99	23.6 (6901)
TGA	57.14	49.40	48.6 (14203)

^aMutations and sequences were taken from the HGMD.¹

^bThe control dataset comprises 1,692 genes listed in the HGMD but for which no nonstop mutations have been recorded to date.

^cBased on a total of 29,210 stop codons associated with annotated human genes. Data from the Transterm database (<http://uther.otago.ac.nz/Transterm.html>)¹⁶

number of missense/nonsense mutations was reported from the *F8* gene (1,217) but only one nonstop *F8* mutation has been reported. Conversely, no missense/nonsense mutations have been recorded for the *HR* gene, even though two nonstop mutations have been identified. Hence we may conclude that the observation that some genes harbour multiple nonstop mutations is unrelated to the number of reported missense and nonsense mutations for those genes.

Gene ontology analysis for genes harbouring nonstop mutations

The Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov/>) was used to identify enriched biological themes within the group of 87 genes harbouring either multiple or single nonstop mutations.¹⁹ A total of 13 terms were found to be significantly enriched ($p < 0.001$, without correction for multiple testing) for single mutations (see Supplementary Table S2). One of the most significantly enriched terms was ‘oxidoreductase’ ($p = 0.005$ after Bonferroni correction), which was associated with 11 of the 67 nonstop mutation-harboring genes identified in the DAVID database.²⁰ Six terms were found to be significantly enriched ($p < 0.001$ without correction for multiple testing) for genes harbouring multiple nonstop mutations (Supplementary Table S3); however, no significant bias in gene function was noted for these genes after correction for multiple testing. A search using all nonstop mutation-containing genes revealed an association with the protein information resource (PIR) term ‘deafness’ ($p = 0.0248$), corresponding to six of 86 sequences, although the biological relevance of this observation remains unclear.

Mutability of the DNA sequence encompassing the mutated stop codons

The dinucleotide mutabilities within the pentanucleotides flanking the naturally mutated stop codons and the randomly mutated HGMD control stop codons were calculated in order to determine whether there was any bias in the mutability of the

various dinucleotides that occur within the three types of stop codon, taking the flanking nucleotides into consideration. A strong positive correlation was noted between the distributions of mutation-harboring dinucleotides and randomly simulated mutations within the stop codons of HGMD control sequences (Pearson’s correlation $r = 0.975$; $p = 8.04 \times 10^{-8}$) with respect to the frequencies of 12 dinucleotides. No significant differences were found in dinucleotide-wise comparisons (Table 2), however, indicating that there is no evidence for a nearest nucleotide-directed bias in stop codon mutability.

Sequence context around stop codons that have been subject to nonstop mutations

In eukaryotic cells, the translational efficiency and readthrough potential of the three different stop

Table 2. The proportion of mutations found within dinucleotides in the mutated stop codon-flanking pentanucleotides as compared with randomly generated HGMD controls

Dinucleotide	Occurrence of nonstop mutations in mutated sequence dataset (%)	Occurrence of random mutations within HGMD control sequences (%)	p value (after correction for multiple testing)
AA	25 (21.00)	348 (20.57)	0.907
AC	6 (5.04)	71 (4.196)	0.636
AG	18 (15.13)	303 (17.91)	0.534
AT	16 (13.44)	238 (14.066)	1.0
CT	23 (19.33)	318 (18.79)	0.903
GG	1 (0.84)	35 (2.07)	NA*
GA	32 (26.89)	424 (25.06)	0.663
GC	1 (0.84)	25 (1.48)	NA*
GT	21 (17.65)	259 (15.31)	0.511
TT	10 (8.4)	155 (9.16)	1.0
TA	36 (30.25)	606 (35.82)	0.235
TG	49 (41.18)	602 (35.58)	0.236

*Sample size of mutated sequences too small to generate p values. (Note that four dinucleotides (CC, CA, CG and TC) cannot occur in conjunction with any stop codon-spanning pentanucleotide and were therefore omitted from this analysis.)

codons have been reported to vary as a consequence of the influence of the surrounding nucleotide sequence.^{21–26} With respect to human gene sequences, Ozawa *et al.* reported that the first three nucleotide positions after the stop codon are highly conserved, with G and A predominating at the +1 position, and C at the +4 position.²⁴ Again in the context of human genes, Liu reported a preponderance of C immediately upstream of the stop codon (at position –1) and G or T at position +1.²⁶ Our HGMD control dataset exhibits similar sequence characteristics to those stop codon datasets reported by Ozawa *et al.*²⁴ and Liu.²⁶ This sequence bias flanking human stop codons represents, in effect, a consensus sequence for the translational termination signal that extends beyond the confines of the stop codon itself. With this in mind, we next examined the flanking sequences of the mutated stop codons in order to ascertain whether the local DNA sequence context could influence the likelihood that the associated nonstop mutations would come to clinical attention.

We first examined the frequencies of six nucleotides on either side of the stop codon in both 87 mutated and 1,692 control sequences. When considering the entire stop codon dataset (which includes sequences flanking the TAA, TAG and TGA stop codons on the 5' side at positions –1 to –6, and on the 3' side at positions +1 to +6), we observed a significant paucity in G at the –2 position ($p = 0.0063$) (Supplementary Table S4). When considering the three types of stop codon separately, there was a significant excess ($p = 0.0016$) of G and a significant paucity of A ($p = 0.0047$) two nucleotides downstream of TAA stop codons (Table 3). Similarly, in the regions flanking TGA stop codons, we noted a significant excess of T at the +6 position ($p = 0.0094$) (Supplementary Table S5). Although it is conceivable that TAA stop codons with a G at +2 and TGA stop codons with a T at +6 may be more prone to mutate than other sequences, we prefer the alternative explanation, that mutations occurring in TAA and TGA stop codons embedded within these sequence contexts are more likely, for whatever reason, to come to clinical attention. No significant difference was

Table 3. Frequency of nucleotides present in regions flanking the mutated TAA stop codon ($N = 40$). Position 0, corresponding to the stop codon, is not shown. Nucleotide frequencies that are significantly higher/lower ($p < 0.01$) in comparison with the HGMD control dataset are shown underlined

Base	–6	–5	–4	–3	–2	–1	1	2	3	4	5	6
A	14	13	7	10	10	5	17	<u>6</u>	11	7	18	11
C	7	9	15	10	13	13	9	10	12	13	9	14
G	8	10	11	5	12	12	11	<u>15</u>	9	9	7	8
T	11	8	7	15	10	10	3	9	8	11	6	7

noted between the flanking regions of mutated and control TAG stop codons (data not shown).

The nucleotide frequencies of the flanking regions of the stop codons that harboured single and multiple mutations were also analysed separately, and compared both with the HGMD control dataset and with each other. Supplementary Table S6 presents the comparison of sequences containing only single mutations with sequences in the HGMD control dataset. These sequences exhibit a significant paucity of G at the –2 ($p = 0.0078$) and –3 ($p = 0.0096$) positions relative to the controls. However, no significant difference was apparent between those sequences harbouring multiple mutations and controls (data not shown).

Sequence context around the next in-frame stop codon downstream of the stop codons that have been subject to nonstop mutations

The DNA sequences around the next downstream in-frame stop codon were analysed using the same method as described above. The regions flanking the next in-frame stop codons located downstream of the mutated stop codons were compared with their counterparts in the HGMD control sequences. This analysis was performed for each of the three codon types (TAA, TAG and TGA) separately and for all the mutated stop codons combined. When analysing all downstream in-frame stop codons together, a significant excess of T was observed at the +6 position ($p = 0.0051$; Supplementary Table S7). When the three types of stop codon were examined separately, the only

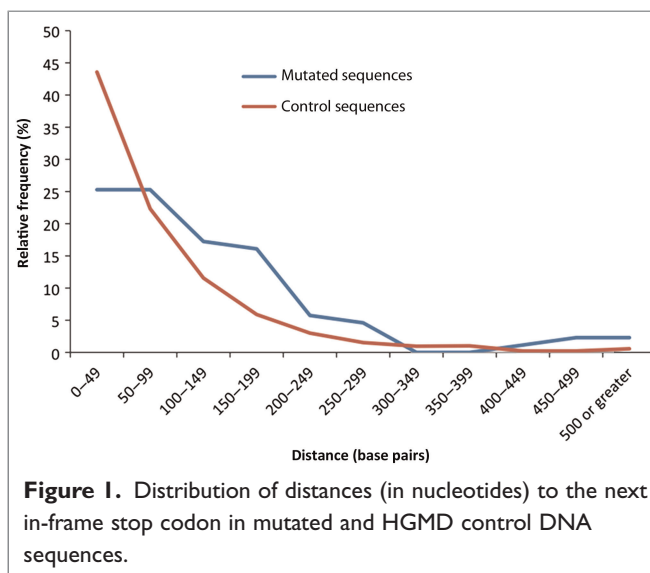
significant difference noted was in the sequences surrounding the next in-frame TGA stop codons, where an excess of C was found at the +6 position ($p = 0.0019$; Supplementary Table S8), as compared with the TGA codons in the control dataset. Taken together, these findings suggest that, in general, there is no obvious difference between the sequences surrounding the next downstream in-frame stop codons and their counterparts in the HGMD control sequences. However, it is possible that the nucleotide occurring at position +6 relative to the downstream alternative in-frame stop codon could influence the likelihood that a given nonstop mutation might come to clinical attention.

The distance to the next stop codon is a key determinant of whether a given nonstop mutation will come to clinical attention

We next explored the possibility that the distance from the mutated stop codon to the next in-frame stop codon downstream might influence the likelihood that a given nonstop mutation would come to clinical attention. We reasoned that the greater the distance between the mutated stop codon and the next viable alternative downstream stop codon, the more likely it would be that the mRNA/protein would be unstable/degraded and hence that the nonstop mutation would give rise to a deleterious and clinically observable phenotype. Conversely, the presence of an alternative in-frame stop codon in the immediate vicinity of the mutated natural stop codon could yield a near-normal or at least ameliorated clinical phenotype. Since such phenotypes would be less likely to come to clinical attention, we might therefore expect there to be a paucity of alternative in-frame stop codons in the immediate vicinity of the mutated stop codons as compared with their counterparts derived from the HGMD control sequences. This was, indeed, what was found when mutated and control sequences were compared. Although a relatively strong correlation was noted between the distributions of the distances (Pearson's correlation 0.75; $p = 0.008$), the number of alternative in-frame stop codons was found to be

significantly lower among the mutated sequences than in the controls, but only in the range 0–49 nucleotides downstream of the mutated stop codon ($p = 7.81 \times 10^{-4}$). This implies that at least some stop codon mutations with alternative stop codons 0–49 nucleotides downstream of the mutated stop codon will not have come to clinical attention, possibly because they will have given rise to stable mRNAs that were (i) not subject to nonstop mRNA decay and (ii) consequently translated into proteins of near-normal length and biological function.

Although the number of in-frame stop codons in the HGMD control dataset approximates to a Zipfian distribution, and steadily decreases with increasing distance from the original stop codon (Figure 1), we noted a significant *excess* (by comparison with the controls) of downstream in-frame stop codons within 150–199 nucleotides of the mutated stop codon ($p = 8.551 \times 10^{-4}$). A significant ($p = 6.558 \times 10^{-6}$) excess of in-frame stop codons within 100–299 nucleotides was also noted as compared with the HGMD controls. One possible explanation could be that the recruitment of these alternative stop codons at an intermediate distance from the mutated stop codon may serve to trigger nonstop mRNA decay, thereby dramatically decreasing the amount of protein product produced and giving rise to a clinical phenotype that is more



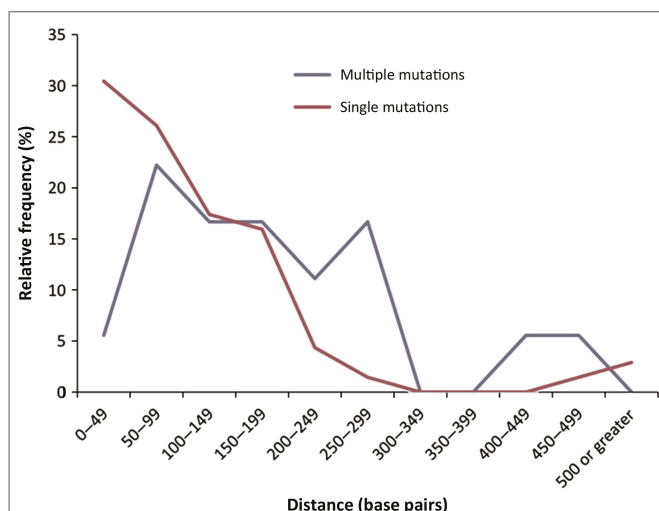


Figure 2. Distribution of distances to the next in-frame stop codon in DNA sequences harbouring single ($N = 69$) and multiple ($N = 18$) mutations.

likely to come to clinical attention. Confirmation or otherwise of this postulate must await the emergence of a clearer understanding of the mechanism of nonstop mRNA decay in mammalian cells.

Figure 2 depicts a comparison of the single ($N = 69$ in 69 genes) and multiple ($N = 18$ in 18 genes) nonstop mutations with respect to the distribution of distances to the next downstream in-frame stop codon in each sequence. If those nonstop mutations which occurred within sequences lacking alternative in-frame stop codons in the range 0–49 nucleotides from the mutated codon did indeed display an increased likelihood of coming to clinical attention, then we might reasonably expect those sequences harbouring multiple nonstop mutations to exhibit an even greater paucity of alternative downstream in-frame stop codons in this size range relative to those sequences harbouring only one nonstop mutation. Although only 18 sequences harboured multiple nonstop mutations (yielding very small sample sizes in each distance category and precluding formal statistical assessment), only one (corresponding to 5.5 per cent of the total number of multiple nonstop mutations) of these sequences bearing multiple nonstop mutations was characterised by an alternative in-frame stop codon within 50 nucleotides downstream of the mutated stop codon, as opposed

to 21 sequences with single mutations (30.9 per cent of the total number of single nonstop mutations) (Figure 2). This finding is therefore wholly compatible with our postulate that nonstop mutations occurring within DNA sequences lacking alternative in-frame stop codons in the immediate vicinity of the mutated stop codon display an increased likelihood of coming to clinical attention, possibly because the resulting extended mRNAs are more likely to be subject to nonstop mRNA decay.

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Table S1. Nonstop mutations recorded in the Human Gene Mutation Database

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence Terminal amino-acids
58	ACTA1	cTAG-CAG	Term-Gln	376	1q21.13	ACTA1	NM_001100.3	1509bp 7 exons	106-1239 TAG	1378-1380 TAA	1465..1470 ATTAAA	tggtccaccgcaaatgctctctagcactcactccactccagcagcag tgc ttc tag = C F *
58	ACTA1	TAG-TGG	Term-Trp								ATTAAA	
58	ACTA1	TAGa-TAT	Term-Tyr								ATTAAA	
326	AIRE	TGAc-TGT	Term-Cys	546	21q22.3	AIRE	NM_000383.2	2257bp 15 exons	128-1765 TGA	1943-1945 TAA	1941..1946 ATTAAA	cggcgccccctccctccctcctgacccagatggccgggacag ccc tcc tga = P S *
336	APOA2	gTGA-AGA	Term-Arg	78	1q21-q23	APOA2	NM_001643.1	473bp 4 exons	59-361 TGA	422-424 TAA	454..459 AATAAA	gaacacagcctgcccaccagctggaagctgcccagaccatgtctt acc cag tga = T Q *
336	APOA2	gTGA-CGA	Term-Arg								AATAAA	
336	APOA2	gTGA-GGA	Term-Gly								AATAAA	
336	APOA2	TGA-TCA	Term-Ser								AATAAA	
353	APRT	TGA-CGA	Term-Arg	181	16q24	APRT	NM_000485.2	807bp 5 exons	36-578 TGA	790-792 TAA	Not identified	tctctctctgcatgatagtagtgcacccacagggctcccagccca tat gag tga = Y E *
353	APRT	TGA-TCA	Term-Ser								AATAAA	
411	ARSB	gTAG-CAG	Term-Gln	534	5q11-q13	ARSB	NM_000046.2	6076bp 9 exons	1287-2888 TAG	3036-3038 TAA	3485..3490 AATAAA	gggtgtggggccttggatctagatctcagggaggctagaaa tgg atg tag = W M *
411	ARSB	TAG-CAG	Term-Gln								AATAAA	
411	ARSB	TAG-CAG	Term-Gln								AATAAA	
411	ARSB	TAG-CAG	Term-Gln								AATAAA	
411	ARSB	TAG-CAG	Term-Gln								AATAAA	
411	ARSB	TAG-CAG	Term-Gln								AATAAA	
435	ASL	TAG-TAC	Term-Tyr	465	7cen-q11.2	ASL	NM_000048.3	1937bp 16 exons	112-1506 TAG	1654-1656 TAA	1528-1533 AATAAA	tactcaggatcagcaggctctaggctctcccaactcctgcccc cag gcc tag = Q A *
435	ASL	TAG-TAC	Term-Tyr								AATAAA	
435	ASL	TAG-TAC	Term-Tyr								AATAAA	
435	ASL	TAG-TAC	Term-Tyr								AATAAA	
435	ASL	TAG-TAC	Term-Tyr								AATAAA	
435	ASL	TAG-TAC	Term-Tyr								AATAAA	

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence
							Number of Exons					Terminal amino-acids
443	ASPA	TAG-TGG	Term-Trp	314	17pter-p13	ASPA	NM_000049.2	1435bp 6 exons	159-1100 TAG	1233-1235 TAA	1364-1369 AATAAA	gattcgcgtctgtttacattga tta cat tag = L H *
472	ATM	gTGA-GGA	Term-Gly	3057	11q22-q23	ATM	NM_000051.3	13147bp 63 exons	386-9556 TGA	9641-9643 TAG	10215-10220 ATTAAA	caggatggaaagcttgggggga tgg gtg tga = W Y *
472	ATM	TGA-TCA	Term-Ser								10514-10519 ATTAAA	
											13129-13134 AATAAA	
477	ATP1A2	cTGA-CGA	Term-Arg	1021	1q21-q23	ATP1A2	NM_000702.2	5496bp 23 exons	133-3195 TGA	3277-3280 TAA	5195-5200 AATAAA	tggaaaggagacatactactctg tac tac tga = Y Y *
50617	ATP6V0A4	gTAG-CAG	Term-Gln	841	7q33-q34	ATP6V0A4	NM_020632.2	3152bp 23 exons	284-2806 TAG	2963-2965 TGA	3039-3044 AATAAA	tggatggcagcggagagagtag gag gag tag = E E *
540	ATP7B	cTGA-CGA	Term-Arg	1466	13q14.3	ATP7B	NM_000053.2	6644bp 21 exons	158-4555 TGA	4556-4558 TGA	3788-3793 ATTAAA	gggatgaggagcagatcatctg tac atc tga = Y *
166379	BBS12	TAG-TAC	Term-Tyr	711	4q27	BBS12	NM_152618.2	3260bp 2 exons	194-2326 TAG	2375-2377 TAA	2379-2384 AATAAA	taacggcttcttattttttgta ttt ttt tag = T L *
120329	CASP12	gTGA-CGA	Term-Arg	125	11q22.3	CASP12	AY358222.1		3-1057 TAA	1064-1066 TAA	1227..1232 AATAAA	ctatctcttctccgggaatta ggg aat taa = G N
846	CASR	aTAA-CAA	Term-Gln	1079	3q13	CASR	NM_000388.2	?bp ? exons	439-3609 TAA	3631-3633 TAG	5831-5836 ATTAAA	agaaaacgtbagaattcataa aat tca taa = N S *

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Chromosome	Codon	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence Terminal amino-acids
1027	CDKN1B	gTAA-CAA	Term-Gln	12p13.1-p12	199	CDKN1B	NM_004064.2	2422bp 3 exons	466-1062 TAA	1240-1242 TGA	1836-1841 ATTAAA	ctcagaagcgaacgtaaacagctcgaattagaatag caa agc <u>taa</u> = Q T *
120329	CFTR	TAG-TGG	Term-Trp	7q31.2	1481	CFTR	NM_000492.3	6132bp 27 exons	133-4575 TAG	4585-4587 TAA	6108..6113 AATAAA	aggtcgaatacaaggcttttagagagcagcaataaagtggac agg ctt <u>tag</u> = R L *
1080	COL1A2	aTAA-CAA	Term-Gln	7q22.1	1277	COL1A2	NM_000089.3	5411bp 52 exons	472-4572 TAA	4585-4587 TAA	4848-4853 AATAAA	tggccagctcttccaataaataaagaactcaatcctaataa ttc aaa <u>taa</u> = F K *
1378	CRYBB1	gTGA-CGA	Term-Arg	22q12.1	253	CRYBB1	NM_001887.3	921bp 6 exons	71-829 TGA	905..907 TAA	903..908 AATAAA	tggccacagagcccccgaagtgaagtcacacctcactctgcta ccc aag <u>tga</u> = P K *
1414	CRYM	TAAa-TAT	Term-Tyr	16p13.11-p12.3	315	CRYM	NM_001888.2	1303bp 9 exons	86-1030 TAA	1043-1045 TGA	1267..1272 AATAAA	attcctggatctggtataataaacaagaagactggtgttg ggt aaa <u>taa</u> = G K *
1428	CTSK	TGAc-TGG	Term-Trp	1q21	330	CTSK	NM_000396.2	1702bp 8 exons	125-1114 TGA	1169-1171 TAA	1650-1655 AATAAA	tggccagcttcccccaagatgtagctccagcagcccaatccat aag atg <u>tga</u> = K M *
1513	CYP2C19	TGAa-TGC	Term-Cys	10q24.1-q24.3	491	CYP2C19	NM_000769.1	1473bp 9 exons	1-1473 TGA	1549-1551 TGA	1617-1622 ATTAAA	agctgtcttcttctctgtctgaaagaagacagatggtctggc cct gtc <u>tga</u> = P V *
1557	DBT	TGA-TTA	Term-Leu	1p31	422	DBT	NM_001918.2	10831bp 11 exons	34-1482 TGA	1501-1503 TGA	Multiple polyA sites	ttatgctactagatctgaatggaagactgataagacattcttg ctg aaa <u>tga</u> = L K *
1629	DHCR7	cTAA-CAA	Term-Gln	11q13.2-q13.5	476	DHCR7	NM_001360.2	2665bp 8 exons	274-1701 TAA	1852-1854 TAA	2099-2105 AATAAA	gccctgctctggaatcttctaaaggacgcctcagggaagaag atc ttc <u>taa</u> = I F *

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	Number of Exons	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence Terminal amino-acids
1717	DOK7	tTGA-CGA	Term-Arg	505	4p16.2	DOK7	NM_173660.3	2566bp	7 exons	71-1585 TGA	2130-2132 TGA	2547-2553 AAATAAA	tcaaggtaaacccccctcttggagccgagatcccgccccc cct cct fga = P P *
285489	EDA	cTAG-CAG	Term-Gln	392	Xq12	EDA	NM_001399.4	5296bp	10 exons	243-1418 TAG	1503-1505 TGA	5251-5256 AAATAAA	tgggtgaagccctgcctcctagattccccccaatttgcctt gca tcc tag = A S *
2110	ETFDH	gTAA-CAA	Term-Gln	618	4q32-q35	ETFDH	NM_004453.2	2349bp	13 exons	333-2186 TAA	2223-2225 TAA	2307-2312 AAATAAA	acctgcttcaatgggaatgtaaacctgagctagcagtttctt gga atg taa = G M *
1896	EYA1	TAAc-TAC	Term-Tyr	593	8q13.3	EYA1	NM_000503.3	4326bp	18 exons	641-2419 TAA	2435-2437 TGA	3014-3020 ATTAAA	ccttggactggagaccctgtaacagctcggcacttgaca tac cag taa = Y L *
2138	F8	cTGA-CGA	Term-Arg	2333	Xq28	F8	NM_000132.2	9030bp	27 exons	172-7227 TGA	7327-7329 TAG	7637-7643 AAATAAA	ggcggacagaccctctactgagggtggcactgacacct ctc tac fga = L Y *
2157	FGB	aTAG-AAG	Term-Lys	462	4q28	FGB	NM_005141.2	1949bp	8 exons	26-1501 TAG	1535-1537 TGA	1649-1655 AAATAAA	ggccttctccacagcaataagttcccccaatcgtagattttt cag caa tag = Q *
2244	FGFR3	gTGA-AGA	Term-Arg	807	4p16.3	FGFR3	NM_000142.2	4093bp	18 exons	40-2460 TGA	2759-2761 TAA	4238-4243 AAATAAA	gcagtgggggtcgcggagcgtggaaggccactggtrcccaaca cgg acg fga = R T *
2261	FGFR3	gTGA-GGA	Term-Gly										
2261	FGFR3	TGA-TCA	Term-Ser										
2261	FGFR3	TGA-TTA	Term-Leu										
2261	FGFR3	TGAa-TGC	Term-Cys										
2261	FGFR3	TGAa-TGG	Term-Trp										
2261	FGFR3	TGAa-TGT	Term-Cys										

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence
							Number of Exons					Terminal amino-acids
2273	FHL1	gTAA-GAA	Term-Glu	281	Xq26	FHL1	NM_001449.3	2398bp 8 exons	209-1051 TAA	1205-1207 TAA	2360..2365 AATAAA	cactgaaaaaagtctccgtggaatctggccaa caagcgcttt gct cag tga = A P *
2261	FKRP	cTGA-AGA	Term-Arg	496	19q13.32	FKRP	NM_024301.3	3349bp 4 exons	2980-1785 TGA	1846-1848 TGA	2489-2494 AATAAA	tgaagcggagagagcggcgtgagccctgataaacctgcctt agc ggc tga = S G *
79147	FM02	tTAG-CAG	Term-Gln	472	1q23-q25	FM02	NM_001460.2	518bp 10 exons	118-1533 TAG	1723-1725 TAG	Multiple polyA sites ?????	tccgacctgcaactctattatgctatcgcctggtggcctgg tcc tat tag = S Y *
2301	FOXE3	gTGA-CGA	Term-Arg	320	1p32	FOXE3	NM_012186.2	2000bp SINGLE exon	245-1204 TGA	1418-1420 TGA	1939-1944 AATAAA	cggggctggagcgtaccctgctgagccctgcgcg-cggcag tac ctg tga = Y L
2301	FOXE3	TGA-TCA	Term-Ser								1954-1959 AATAAA	
2294	FOXFI	gTGA-CGA	Term-Arg	380	16q24	FOXFI	NM_001451.2	2579bp 3 exons	44-1183 TGA	1400-1402 TAG	3218-3223 AATAAA	acatcaagccttgcgtgagtgtagggctccgcgcagccct gtg ag tga = V M *
2327	FOXHI	gTGA-CGA	Term-Arg	366	8q24.3	FOXHI	NM_003923.1	1793bp 3 exons	580-1677 TGA	1684-1686 TAA	Not found	tgtctctctggcagcctgaggcctcttaagacaggccca agc ctg tga = S L *
8928	FUCAI	gTAA-AAA	Term-Lys	462	1p34	FUCAI	NM_000147.3	2095bp 8 exons	46-1446 TAA	1681-1683 TGA	1575-1581 AATAAA	taaagctgacagagtggaagtaatcatttggagcgaagaaga gtg aag taa = V K *
2517	GALT	cTGA-CGA	Term-Arg	380	9p13	GALT	NM_000155.2	1347bp 11 exons	68-1207 TGA	1352-1354 TAA	1315-1320 AATAAA	gggaagacaaccatcgcctggaccacccgacacaggccct atc gcc tga = I A *
2592	GALT	TGAc-TGC	Term-Cys									
2623	GATAI	aTGA-CGA	Term-Arg	414	Xp11.23	GATAI	NM_002049.2	1522bp 6 exons	113-1354 TGA	1475-1477 TAA	1478-1484 AATAAA	gtggctcctcagctcatgaggcacaagatggcct agc tca tga = S S *
2592	GCDH	TGAG-TGG	Term-Trp	439	19p13.2	GCDH	NM_000159.2	1839bp 12 exons	78-1394 TGA	1473-1475 TGA	1802..1807 AATAAA	agggcttcaaggccagcaagctggagcgcctccatcaggggcccg agc aag tga = S K *

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence
2639	GCHI	cTGA-CGA	Term-Arg	251	14q22.1-q22.2	GCHI	NM_000161.2	2941bp 6 exons	162-914 TGA	1017-1019 TGA	Multiple polyA sites 2896-2901 ATTAAA	tcctgactctcattaggagctgagctcttcctcctcagtggtgtgc agg agc tga = R S *
2645	GCK	gTGA-CGA	Term-Arg	466	7p15.3-p15.1	GCK	NM_000162.2	2759bp 10 exons	487-1884 TGA	2314-2316 TGA	2724-2729 ATTAAA	aggcctgtatgctgggccaagtgagagcagtggtgcgcaagcagcag ggc cag tga = G Q *
55806	HR	cTAG-CAG	Term-Gln	35	8p21.2	HR	NM_005144.3	4981bp 19 exons	131-3700 TAG	4151-4153 TGA	4311-4316 ATTAAA	caggagccaaatagaggatgctaggg gcc aaa tag = A K *
	HR	TAG-TGG	Term-Trp								4952-4957 ATTAAA	
2643	HBA2	TAA-gTAT	Term-Tyr	142	16p13.3	HBA2	NM_000517.3	575bp 3 exons	38-466 TAA	557-559 TAA	555-560 AAATAAA	tgtgacctccaaataccgttaagctggagcctcagctgagcgt tac cgt taa = Y R *
3040	HBA2	tTAA-AAA	Term-Lys									
3040	HBA2	tTAA-CAA	Term-Gln									
3040	HBA2	tTAA-GAA	Term-Glu									
3040	HBA2	tTAA-TCA	Term-Ser									
3081	HGD	tTGA-CGA	Term-Arg	446	3q13.33	HGD	NM_000187.2	1920bp 14 exons	371-1708 TGA	1778-1780 TAG	1892-1898 AAATAAA	ccagcagaacctaatgtgagactggaacattgctaccataa cct aat tga = P N *
3284	HSD3B2	TGA-tTGC	Term-Cys	373	1p13.1	HSD3B2	NM_000198.2	1669bp 4 exons	143-1261 TGA	1544-1546 TGA	1649-1654 AAATAAA	ccctgagctccaagaccagctgatttttaaggatgacagagatgt act cag tga = T Q *
3425	IDUA	aTGA-GGA	Term-Gly	654	4p16.3	IDUA	NM_000203.3	2203bp 14 exons	89-2050 TGA	2231-2233 TGA	2145-2150 AAATATA	ccccatccccgggcaatccatgagctgctgctgagcccgagtg aat cca tga = N P *
3425	IDUA	TGAg-TGT	Term-Cys									
8517	IKBK	TAG-TGG	Term-Trp	420	Xq28	IKBK	NM_001099856.1	2073bp 10 exons	225-1483 TAG	1563-1565 TAG	2049-2054 AGTAAA	atgctatgagctgattgtagggccggccagtgcaaggcca att gag tag = I E *

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence
							Number of Exons					Terminal amino-acids
9445	ITM2B	cTGA-AGA	Term-Arg	267	13q14.3	ITM2B	NM_021999.3	1870bp 6 exons	1874-987 TGA	1018-1020 TAA	1131-1136 ATTAAA 1440-1445 ATTAAA 1664-1669 AATAAA 1785-1790 AATAAA 1834-1839 ATTAAA	tggaactttaatttcttfgaacagctcaagaanaacattat tgt tct tga = C K *
169522	KCNV2	cTAG-TAT	Term-Tyr	546	9p24.2	KCNV2	NM_133497.2	1882bp 2 exons	215-1852 TAG	2031-2033 TAG	2142-2147 AATAAA	tcacccaagaagaagatttagtattttataggaatggtggc gag aat tag = E N *
169522	KCNV2	cTAG-CAG	Term-Gln									
84634	KISS1R	cTGA-AGA	Term-Arg	399	19p13.3	KISS1R	NM_032511.4	1607bp 5 exons	146-1342 TGA	1839-1841 TAA	1554-1559 ATTAAA	ggaggacaagccctctct tga gcgaccctggfgggaatccg cct ctc tga = P L *
3914	LAMB3	TGA-tTGG	Term-Trp	1173	1q32	LAMB3	NM_000228.2	4093bp 23 exons	145-3663 TGA	3829-3831 TGA	4008-4013 AATGAA 4020-4025 AATAAA	tctactatgccaccctgcaag tga tgctacagcttcagcccg tgc aag tga = C K *
9388	LIPG	cTGA-CGA	Term-Arg	501	18q21.1	LIPG	NM_006033.2	4143bp ? exons	253-1755 TGA	1900-1902 TGA	4094-4099 ATTAAA 4118-4123 AATAAA	actgtagcttctc tga ggggtccgggcaagctcttg ctt ccc tga = L P *
4143	MAT1A	TAGa-TAT	Term-Tyr	396	10q22	MAT1A	NM_000429.2	3419bp 9 exons	256-1443 TAG	1645-1647 TAA	3382-3387 AATAAA	ttcccaggagctgtatt tga gacagggggagctgggccc gta ttt tag = V F *
4159	MC3R	TAG-TCG	Term-Ser	361	20q13.2-q13.3	MC3R	NM_019888.2	1112bp 1 exon	1-1083 TAG	1102-1104 TGA	Not found	gcaacggcatgaactgggata tga gacagggccatggaatg ttg gga tag = L G *
64087	MCCC2	gTAA-CAA	Term-Gln	564	5q12-q13	MCCC2	NM_022132.3	2329bp 17 exons	100-1791 TAA	17987-1800 TAA	1796-1801 AATAAA	acttcggatcttcagag tga ctggaataaaggatgttttc agg aag taa = R M *

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	Number of Exons	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence
5080	MECP2	cTGA-TGG	Term-Trp	487	Xq28	MECP2	NM_004992.2	10241bp	4 exons	227-1687	1766-1768	1790-1795	cctgtgaccgagagtagctgacttatacagggagggattgc gtt agc tga = V S *
5080	MECP2	cTGA-CGA	Term-Arg							TGA	TGA	AATAAA	
5080	MECP2	cTGA-TTA	Term-Leu									7191-7196	
5080	MECP2	cTGA-TGC	Term-Cys									7300-7305	TATAAA
												9490-9495	AATAAA
													AATAAA
4338	MOCS2	TAA-t-TAC	Term-Tyr	189	5q11	MOCS2	NM_004531.3	1347bp	8 exons	40-793	845-847	1238-1243	gctttgggcatccaacagttgactcactctatgcttttttagagca aac agt taa = N S *
										TAA	TGA	ATTAATA	
												1289-1294	ATTAATA
												1299-1304	ATTAATA
													AATAAA
4524	MTHFR	TGA-TCA	Term-Ser	657	1p36.3	MTHFR	NM_005957.3	7105bp	12 exons	185-2155	2303-2305	3833-3838	cgagagaaacggaggctccatgagccctcgctcctgacccctg gct cca tga = A P *
										TGA	TGA	?????	
												7086-7091	AATAAA
55651	NHP2	aTGA-AGA	Term-Arg	154	5q35.3	NHP2	NM_017838.3	867bp	4 exons	144-605	756-758	802-805	agtcctcctccctccctccctccctccctccctccctccctccctg ccc cta tga = P L *
										TGA	TGA	ACTAAA	
												836-841	AGTAAA
4878	NPPA	cTGA-CGA	Term-Arg	152	1p36.21	NPPA	NM_006172.2	840bp	3 exons	95-550	554-556	768-773	ctggttctcttgcagactgagagataaacagcagcagggaggac cag tac tga = Q Y *
										TGA	TAA	ATTAATA	
												819-824	AATAAA
190	NR0B1	aTAA-GAA	Term-Glu	471	Xp21.3-p21.2	NR0B1	NM_000475.3	1555bp	2 exons	13-1424	1447-1479	1475-1480	aaatgctctgatacaagataaaagrtcagtgaggccacaaga aag ata taa = K I *
										TAA	TAA	AAATAAA	
												1514-1519	AAATAAA
													AATAAA
4939	OAS2	TAG-TGG	Term-Trp	720	12q24.2	OAS2	NM_016817.2	3539bp	11 exons	141-2300	2322-2324	3015-3020	ataattctaaagaactctctgagatcatctggcaatcgctt aac ttc tag = N F *
										TAG	TAA	AAATAAA	
												3340-3345	AAATAAA
												3513-3518	AAATAAA
													AATAAA

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA Acc Num (Longest)	Transcript size	Number of Exons	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence	Terminal amino-acids
4976	OPA1	TAAa-TAC	Term-Tyr	961	3q28-q29	OPA1	NM_015560.1	5864bp 31 exons	56-2938	TGA	2975-29771	3046-3051	aagctcttcatcagggaaataaaataaagtgagtaaaaaattct gag aaa <u>taa</u> = E K *	AATAAA ATATAA
5009	OTC	TGAa-TGG	Term-Trp	355	Xp21.1	OTC	NM_000531.4	1647bp 10 exons	215-1279	TGA	1319-1321	1365-1370	agctcccaagcccaaatcttgaatggtgtactctgtcaaga aaa ttt <u>tga</u> = K F *	AATAAA AATAAA
5080	PAX6	TAA-TTA	Term-Leu	423	11p13	PAX6	NM_000280.2	2816bp 15 exons	513-1781	TAA	1821-1823	2269-2274	aatatcgcccaagattacgtaa aaaaaaaaaaaaaaaaaaaggaagaaaa tta cag <u>taa</u> = L Q *	AATAAA AATAAA
5189	PEX1	aTAA-CAA	Term-Gln	1284	7q21.2	PEX1	NM_000466.2	4390bp 24 exons	97-3948	TAA	4030-4032	4261-4266	gacagaagtaacttagcataaataatctcttttggatt tta gca <u>taa</u> = L A *	AATAAA AATAAA
8929	PHOX2B	TGAa-TGG	Term-Trp	315	4p12	PHOX2B	NM_003924.2	3033bp 3 exons	361-1305	TGA	1426-1428	1452-1457	tagtgaagcagcagctgtctgtatctggaatctctctggcggcg atg ttc <u>tga</u> = M F *	AATAAA AATAAA AATAAA AATAAA AATAAA
8929	PHOX2B	TGAa-TGC	Term-Cys											
55163	PNPO	tTAA-CAA	Term-Gln	262	17q21.32	PNPO	NM_018129.2	3482bp 7 exons	154-939	TAA	1021-1023	1405-1410	tcctatgagcctgcaccttaacctcgggacctctggccca gca cct <u>taa</u> = A P *	ATATAA ATATAA ATATAA 3438-3443 AATAAA
5627	PROS1	TAAg-TAT	Term-Tyr	636	3q11.2	PROS1	NM_000313.1	3309bp 15 exons	147-2177	TAA	2217-2219	2636-2641	ggaaaaagcaaaagaatccttaagcactcttctctcttat aat tct <u>taa</u> = N A *	ATATAA ATATAA ATATAA 3289-3294 AATAAA

Continued

Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence
							Number of Exons					Terminal amino-acids
10594	PRPF8	cTGA-CGA	Term-Arg	2336	17p13.3	PRPF8	NM_006445.3	731 bp 43 exons	115-7122 TGA	7243-7245 TGA	7261-7266 AATAAA 7274-7279 AATAAA	atcggggaccgtatgctcgtgacccgtttccctcctcgtct tat gcc tga = Y A *
5744	PTHLH	TGAa-TGG	Term-Trp	178	12p12.1-p11.2	PTHLH	NM_198965.1	133 bp 5 exons	323-856 TGA	1013-1015 TGA	1304-1309 AATAAA	ttcacggagcattgaaattttcagcagagccttc agg cat tga = R H
10111	RAD50	TAAa-TAT	Term-Tyr	1313	5q31	RAD50	NM_005732.2	589 bp 25 exons	388-4326 TAA	4522-4524 TGA	5836-5841 AATAAA	tgggattcaatgttcattataaaaaatcccaagatttaaag ggt cat taa = V H *
6066	RHCE	TAAg-TAC	Term-Tyr	418	1p36.11	RHCE	NM_020485.3	1635 bp 9 exons	87-1340 TAA	1416-1418 TGA	1482-1487 AATAAA 1490-1495 AATAAA 1536-1541 AATAAA 1596-1601 AATAAA	attgctgtggattttaagcaaaagcatccaagaaaaa gga ttt taa = G F *
6010	RHO	cTAA-CAA	Term-Gln	349	3q21-q24	RHO	NM_000539.2	2768 bp 5 exons	96-1142 TAA	1293-1295 TAA	1239-1244 AATGAA 1506-1511 AATAAA 1659-1664 TATAAA 2563-2568 AATAAA	cgaagccaggcccccggccctaaagaccctcctaggactctgag cgc gcc taa = P A *
860	RUNX2	TGA-TCA	Term-Ser	522	6p21	RUNX2	NM_001024630.2	5572 bp 9 exons	7-1776 TGA	1853-1855 TAG	2761-2666 AATAAA 3073-3078 AATAAA 3892-3897 AATAAA 4183-4188 AATAAA 4448-4453 AATAAA 4591-4596 AATAAA	aatcgtttggaccacatattgaaattcctcagcagggccca cca tat tga = P Y

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Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	Number of Exons	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence	Terminal amino-acids
710	SERPING1	cTGA-AGA	Term-Arg	479	11q12-q13.1	SERPING1	NM_000062.2	1984bp	8 exons	192-1694 TGA	1830-1832 TGA	1940-1945 AAATAAA ATATAA	gagtatatagaccagggcctgagaccctcagatcagatttag agg gcc tga = R A *	
4068	SH2D1A	aTGA-AGA	Term-Arg	129	Xq25-q26	SH2D1A	NM_002351.2	2507bp	4 exons	346-732 TGA	766-768 TAA	738-743 AAATAAA 1036-1041 AAATAAA	atgctctgctgaaagcccccctatgaaagaaataaacaacctgtg gcc cca tga = A P *	
6473	SHOX	cTGA-CGA	Term-Arg	293	Xp22.33	SHOXa	NM_000451.3	3757bp	6 exons	692-1570 TGA	1712-1715 TAG	2486-2491 ATATAA	gcgaggccctggggctctgacccgcgcgagccc ggg ctc tga = G L *	
6473	SHOX	aTGA-CGA	Term-Arg	226		SHOXb	NM_006883.2	1951bp	6 exons	692-1369 TGA	1433-1436 TAG	Not found		
5172	SLC26A4	TGAa-TGG	Term-Trp	781	7q31	SLC26A4	NM_000441.1	4930bp	21 exons	225-2567 TGA	2691-2693 TAA	2719-2724 AAATAAA 3014-3019 AAATAAA 3038-3043 AAATAAA 3066-3071 AAATAAA 3229-3234 AAATAAA	ctatgctatcattgcatctctgaaagggcttgaggagctc gca tcc tga = A S *	
54977	SLC25A38	cTGA-CGA	Term-Arg	305	3p22.1	SLC25A38	NM_017875.2	2124bp	7 exons	402-1316 TGA	1398-1400 TAA	1897-1902 AAATAAA 1965-1670 ATATAA 2092-2097 AAATAAA	gggcctgaaagccttgacccaagaaggagctgg aag tcc tga = K S *	
6663	SOX10	cTAA-TAC	Term-Tyr	467	22q13.1	SOX10	NM_006941.3	2882bp	4 exons	279-1679 TAA	1935-1937 TGA	2840-2845 AAATAAA 2846-2851 ATATAA	atagacactgtccggccctaaaggggcctgtgcacca cgg ccc taa = R P *	
6663	SOX10	cTAA-AAA	Term-Lys											
6716	SRD5A2	TAA-TCA	Term-Ser	255	2p23	SRD5A2	NM_000348.3	2446bp	5 exons	72..836 TAA	918-920 TAA	846-851 ATATAA 1235-1240 ATATAA 2426-2431 ATATAA	cccttattccattcatcttttaaaaggaccataataaaggga atc ttt taa = I F *	

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Table S1. Continued

Entrez Gene ID	Gene	Base change	Amino acid change	Codon	Chromosome	Gene	Ref_Seq mRNA (Longest) Transcript size	Acc Num	Number of Exons	CDS	Next STOP codon	polyA signals	Flanking nucleotide sequence
7170	TPM3	TAA-TCA	Term-Ser	286	1q21.2	TPM3	NM_152263.2	7116bp	12 exons	116-973 TAA	1142-1144 TAA	1140-1145 AATAAA ATATAA	tccttacttttcaacagagataaattatcacagcttctctcgtg tac aga <u>taa</u> = Y R *
7454	WAS	cTGA-AGA	Term-Arg	503	Xp11.4-p11.21	WAS	NM_000377.1	1806bp	12 exons	35-1543 TGA	1805-1807 TAA	1777-1782 AATAAA	aagatgatgaatgggagactgaggcgtgagttacttctgctg gat gac <u>tga</u> = D D *
7454	WAS	cTGA-CGA	Term-Arg										
7454	WAS	TGA-TCA	Term-Ser										
7490	WT1	TGAG-TGG	Term-Trp	450	11p13	WT1	NM_024424.2	3020bp	10 exons	197-1741 TGA	1805-1807 TGA	2206-2211 AATAAA 3002-3007 AATAAA	ccaaactccagctggcgttctgagggtctccctcggggacag gcg ctt <u>tga</u> = A L *

Table S2. Major enriched ($p < 0.001$) categories for genes harbouring single mutations in stop codons

Category	Term	Count	%	p value	Genes
SP_PIR_KEYWORDS	Oxidoreductase	11	16.42	2.03E-05	<i>HSD3B2, DBT, GCDH, MTHFR, CYP2C19, DHCR7, FMO2, ETFDH, HGD, PNPO, SRD5A2</i>
GOTERM_BP_FAT	GO:0044271 ~ nitrogen compound biosynthetic process	9	13.43	1.40E-04	<i>MOCS2, OTC, SLC25A38, ATP1A2, ASL, ATP6V0A4, NPPA, ATP7B, GCHI</i>
GOTERM_BP_FAT	GO:0008015 ~ blood circulation	7	10.45	2.41E-04	<i>MTHFR, COL1A2, SERPING1, CFTR, ATP1A2, NPPA, GCHI</i>
GOTERM_BP_FAT	GO:0003013 ~ circulatory system process	7	10.45	2.41E-04	<i>MTHFR, COL1A2, SERPING1, CFTR, ATP1A2, NPPA, GCHI</i>
GOTERM_MF_FAT	GO:0050662 ~ coenzyme binding	7	10.5	2.59E-04	<i>DBT, GCDH, FMO2, ETFDH, PNPO, CRYM, GCHI</i>
SP_PIR_KEYWORDS	Blood coagulation	4	5.97	4.62E-04	<i>FGB, F8, SERPING1, PROS1</i>
SP_PIR_KEYWORDS	Flavoprotein	5	7.46	5.00E-04	<i>GCDH, MTHFR, FMO2, ETFDH, PNPO</i>
GOTERM_CC_FAT	GO:0031093 ~ platelet alpha granule lumen	4	5.97	6.78E-04	<i>FGB, F8, SERPING1, PROS1</i>
GOTERM_BP_FAT	GO:0006694 ~ steroid biosynthetic process	5	7.46	6.92E-04	<i>HSD3B2, DHCR7, CFTR, SRD5A2, NR0B1</i>
GOTERM_BP_FAT	GO:0042592 ~ homeostatic process	12	17.91	7.17E-04	<i>PTH1LH, SLC26A4, CTSK, CASR, OTC, IKBKG, SLC25A38, LIPG, ATP1A2, ATP6V0A4, RAD50, ATP7B</i>
GOTERM_BP_FAT	GO:0055114 ~ oxidation reduction	11	16.42	7.76E-04	<i>HSD3B2, GCDH, MTHFR, CYP2C19, DHCR7, FMO2, ETFDH, F8, HGD, PNPO, SRD5A2</i>
GOTERM_CC_FAT	GO:0060205 ~ cytoplasmic membrane-bounded vesicle lumen	4	5.97	8.35E-04	<i>FGB, F8, SERPING1, PROS1</i>
GOTERM_CC_FAT	GO:0031983 ~ vesicle lumen	4	5.97	9.52E-04	<i>FGB, F8, SERPING1, PROS1</i>

Table S3. Major enriched ($p < 0.001$) categories for genes harbouring multiple mutations in stop codons

Category	Term	Count	%	p value	Genes
SP_PIR_KEYWORDS	DNA-binding	8	42.11	9.77E-04	SOX10, PHOX2B, MECP2, PAX6, HR, SHOX, ATM, FOXE3
SP_PIR_KEYWORDS	Peters' anomaly	2	10.53	0.0047	PAX6, FOXE3
SP_PIR_KEYWORDS	Transcription regulation	7	36.84	0.0082	SOX10, PHOX2B, MECP2, PAX6, HR, SHOX, FOXE3
GOTERM_MF_FAT	GO:0043565 ~ sequence-specific DNA binding	5	26.32	0.0086	SOX10, PHOX2B, PAX6, SHOX, FOXE3
GOTERM_MF_FAT	GO:0003700 ~ transcription factor activity	6	31.58	0.0089	SOX10, PHOX2B, PAX6, HR, SHOX, FOXE3
SP_PIR_KEYWORDS	Transcription	7	36.84	0.0092	SOX10, PHOX2B, MECP2, PAX6, HR, SHOX, FOXE3

Table S4. Frequency of nucleotides present in regions flanking the 87 mutated stop codons. Position 0, corresponding to the stop codon, is not shown. Nucleotide frequencies that are significantly higher/lower ($p < 0.01$) in comparison to the HGMD control dataset are shown underlined

Base	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6
A	25	25	12	25	29	16	31	20	19	13	28	20
C	18	20	27	26	24	27	15	26	26	25	22	28
G	24	23	28	14	<u>7</u>	24	28	28	19	21	21	19
T	20	19	20	22	27		20	13	23	28	16	20

Table S5. Frequency of nucleotides present in regions flanking the mutated TGA stop codon ($N = 35$). Position 0 corresponding to the stop codon is not shown. Nucleotide frequencies that are significantly higher/lower ($p < 0.01$) in comparison to the HGMD control dataset are shown in bold

Base	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6
A	9	9	4	12	12	9	12	9	8	6	10	6
C	7	8	10	13	10	11	4	12	8	9	8	9
G	12	10	11	7	5	9	13	10	9	9	8	8
T	7	8	10	3	8	6	6	4	10	11	9	12

Table S6. Frequency of nucleotides occurring within regions flanking mutated stop codons harbouring single nonstop mutations. Position 0 corresponding to the stop codon is not shown. Frequencies which are significantly higher/lower ($p < 0.01$) in comparison with corresponding HGMD controls are shown underlined

Base	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6
A	21	19	11	21	21	14	26	15	16	11	23	16
C	14	17	19	19	19	19	11	19	22	21	18	23
G	19	18	22	<u>9</u>	<u>5</u>	17	21	23	13	14	14	14
T	14	14	16	19	23	18	10	11	17	22	13	15

Table S7. Frequencies of nucleotides flanking the next downstream in-frame stop codon in mutated sequences. Position 0, corresponding to the stop codon, is not shown. Frequencies which are significantly higher/lower ($p < 0.01$) in comparison with the corresponding HGMD controls are shown underlined

Base	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6
A	9	10	14	16	8	9	13	10	17	14	16	15
C	13	11	7	7	12	17	12	17	11	16	9	15
G	8	15	11	9	8	10	12	12	9	9	10	12
T	16	10	14	15	19	11	11	9	10	8	12	<u>5</u>

Table S8. Frequencies of nucleotides flanking the next downstream in-frame TGA stop codon. Position 0, corresponding to the stop codon, is not shown. Frequencies which are significantly higher/lower ($p < 0.01$) in comparison with the corresponding HGMD controls are shown in bold

Base	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6
A	4	6	9	9	3	1	5	6	7	6	9	4
C	7	8	4	3	6	11	7	10	8	10	5	12
G	6	8	6	4	5	7	8	4	4	4	4	6
T	8	3	6	9	11	6	6	6	6	5	7	3