Supplementary Appendix

Supplement to: Chovanec et al. **Genetically determining individualized clinical reference ranges for the biomarker tryptase can limit unnecessary procedures and unmask myeloid neoplasms**

Table of Contents:

SUPPLEMENTAL METHODS

Tryptase isoform re-alignment script

```
import pysam
import glob
import pandas as pd
import argparse

parser = argparse.ArgumentParser(description='Create a CSV file for further processing')

parser.add_argument('--bam_folder', type=str, nargs=1, required=True,
                    help='The directory containing the bam files.')
parser.add_argument('--source', type=str, nargs=1, required=True,
                    help='The source of the bam files e.g. AML')
parser.add_argument('--roi_start', type=int, nargs=1, required=True,
                    help='The 0 based start position of ROI')
parser.add_argument('--roi_end', type=int, nargs=1, required=True,
                    help='The 0 based end position of ROI')
parser.add_argument('--output_name', type=str, nargs=1, required=True,
                    help='The name of the output file')
args = parser.parse_args()

print (args)


def get_files(directory_path):
    """
    Return a list containing all the files matching a pattern in \
    the given directory

    """

    print ('{directory_path}/*.sorted.bam'.format(
                    directory_path=directory_path))
    file_list  = glob.glob('{directory_path}/*.sorted.bam'.format(
                    directory_path=directory_path))

    return file_list

def get_read_count(df):
    """
    Get the read count in a specific bam file.

    """

    return int(pysam.view("-c", df['file_location']))

def get_transcript_count(df, transcript_name):

    """
    Return the count of the transcript i.e how many of each of \
    the three transcripts are in there.
    Note - Does not look at the quality of the alignment.
    """

    sam_file_location = df['file_location']

    samfile = pysam.AlignmentFile(sam_file_location, "rb")

    count = 0

    for read in samfile:

        if read is not None and read.reference_name == transcript_name:

            count = count + 1

    return count

def get_zero_edit_distance_count(df, transcript_name):

    """
    For a goven transcript e.g. Alpha_GEX_64k_HEX get how many of \
    the matches have an NM tag of zero
    i.e. the match was exact.

    """

    sam_file_location = df['file_location']

    samfile = pysam.AlignmentFile(sam_file_location, "rb")

    count = 0

    for read in samfile:

        if read is not None and read.reference_name == transcript_name:

            edit_distance = read.get_tag('NM')

            if edit_distance == 0:

                count = count + 1

    return count

def get_transcript_read_count_filtered(df, transcript_name, start, end):

    """
    Count hits which cover the bit of the reference we are interested in.

    start = 0 based position in reference the alignment must start on or before.
    end = 0 based position that the alignment must end on or before.

    """

    sam_file_location = df['file_location']

    samfile = pysam.AlignmentFile(sam_file_location, "rb")

    iter = samfile.fetch(transcript_name, start, end)

    count =0

    for read in iter:

        if read.reference_start <=start and read.reference_end >= end:

            count = count +1

    return count

def get_transcript_read_count_filtered_exact(df, transcript_name, start, end):

    """
    Count hits which cover the bit of the reference we are interested in \
    and which are exact.

    That is - do they cross position 0 - 44 of the transcript \
    (given by transcipt_name) and \
    have an edit distance from the reference of 0.

    start = 0 based position in reference the alignment must start on or before.
    end = 0 based position that the alignment must end on or before.

    """

    sam_file_location = df['file_location']

    samfile = pysam.AlignmentFile(sam_file_location, "rb")

    iter = samfile.fetch(transcript_name, start, end)

    count =0

    for read in iter:

        if read.reference_start <=start and read.reference_end >=end:

            edit_distance = read.get_tag('NM')

            if edit_distance == 0:

                count = count + 1

    return count


results = get_files(args.bam_folder[0])
file_names = [x.split('/')[len(x.split('/'))-1] for x in results]

#Create Dataframe
df = pd.DataFrame(index=file_names)

df['file_location'] = results
df['source'] = args.source[0]


#Add total-count column
df['alignment_count'] = df.apply(get_read_count, axis=1)
print ('Added total alignment count')



#Count the number of reads aligned to each reference transcript
df['alpha_wt_count'] = df.apply(get_transcript_count, axis=1, args=['Alpha_GEX_64k_HEX'])
df['alpha_dup_count'] = df.apply(get_transcript_count, axis=1, args=['Alpha_GEX_79k_dup_FAM'])
df['beta_count'] = df.apply(get_transcript_count, axis=1, args=['BETA_new_GEX_FAM'])
print ('Added alignment count for each transcript')


#Number of exact read alignments for each reference transcript
df['alpha_wt_zero_edit_count'] = df.apply(get_zero_edit_distance_count, axis=1, args=['Alpha_GEX_64k_HEX'])
df['alpha_dup_zero_edit_count'] = df.apply(get_zero_edit_distance_count, axis=1, args=['Alpha_GEX_79k_dup_FAM'])
df['beta_zero_edit_count'] = df.apply(get_zero_edit_distance_count, axis=1, args=['BETA_new_GEX_FAM'])
print ('Added exact alignment count for each transcript')

# Number of alignments that span our area of interest

start = args.roi_start[0]
end = args.roi_end[0]

df['alpha_read_covers_snps_count'] = df.apply(get_transcript_read_count_filtered,
                                              axis=1,
                                              args=['Alpha_GEX_64k_HEX', start,end])
df['alpha_dup_read_covers_snps_count'] = df.apply(get_transcript_read_count_filtered,
                                              axis=1,
                                              args=['Alpha_GEX_79k_dup_FAM', start,end])
df['beta_read_covers_snps_count'] = df.apply(get_transcript_read_count_filtered,
                                              axis=1,
                                              args=['BETA_new_GEX_FAM', start,end])
print ('Added alignment count for each transcript within ROI')


# Number of alignments that span our area of interest and are exact
df['alpha_read_covers_snps_count_exact'] = df.apply(get_transcript_read_count_filtered_exact,
                                              axis=1,
                                              args=['Alpha_GEX_64k_HEX', start,end])

df['alpha_dup_read_covers_snps_count_exact'] = df.apply(get_transcript_read_count_filtered_exact,
                                              axis=1,
                                              args=['Alpha_GEX_79k_dup_FAM', start,end])

df['beta_read_covers_snps_count_exact'] = df.apply(get_transcript_read_count_filtered_exact,
                                              axis=1,
                                              args=['BETA_new_GEX_FAM', start,end])

print ('Added exact alignment count for each transcript within ROI')

df.to_csv(args.output_name[0])
print ('Created CSV')
```

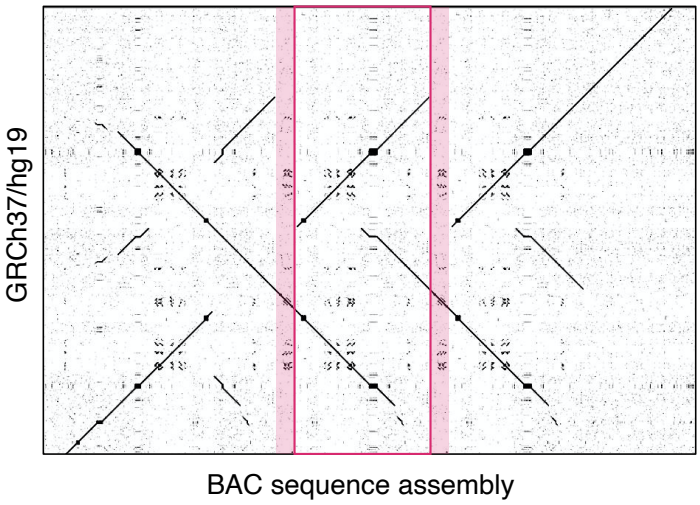


Figure S1. Alignment of the tryptase locus in hereditary alpha tryptasemia. Dot-plot of the assembled tryptase locus from an individual with a *TPSAB1* duplication (BAC clone assembly, X-axis) with the human reference sequence GRCh37/hg19 chr16:1,270,000-1,315,000 (Y-axis).

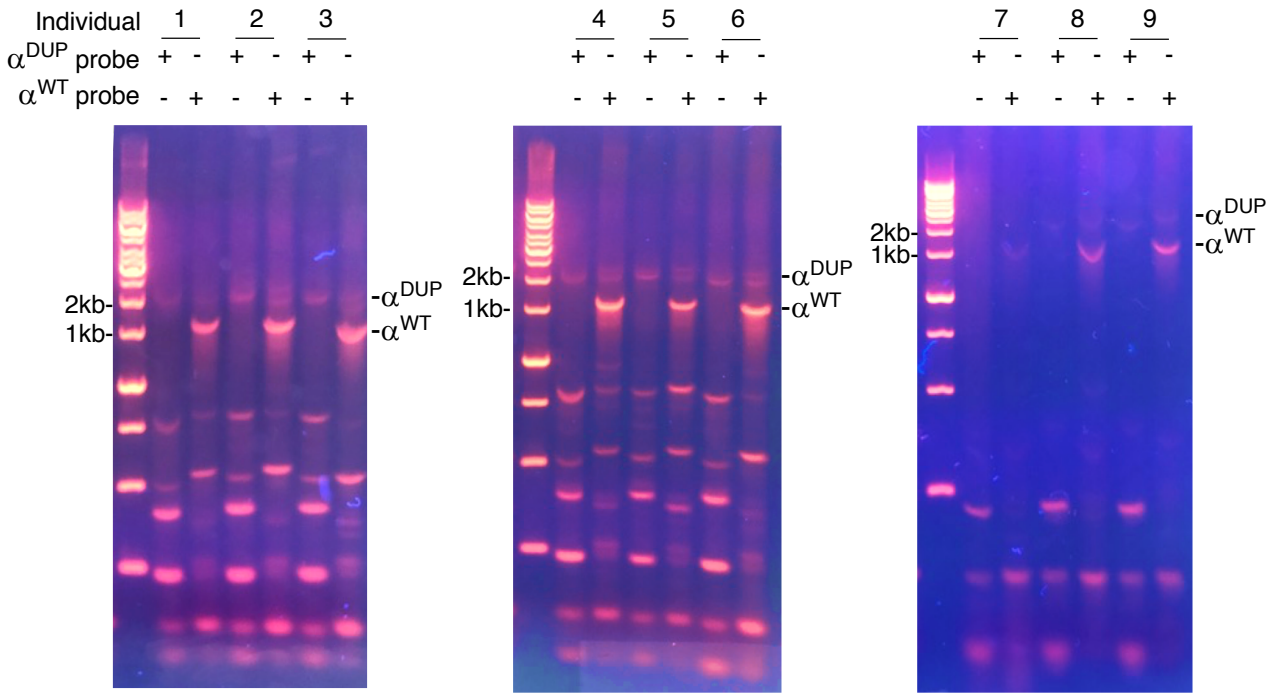


Figure S2. Gel electrophoresis of alpha-tryptase linked promoters. Genomic DNA from nine individuals with hereditary alpha tryptasemia was amplified with duplicated (α^{DUP}) and wild-type alpha-tryptase (α^{WT}) specific forward primers paired with an alpha-tryptase specific reverse primer**.** The largest molecular weight bands of approximately 1 and 2 kilobases (kB) respectively correspond to the full-length proximal coding sequences and linked promoters.

```
CLUSTAL O(1.2.4) multiple sequence alignment

aDUP_CACCT_BAC          GGGGCCGGGGGTGAGACCATGGGGAGCTGGGCTGGGGCTGGGGGTGAGACCATGGGGAGC    60
aDUP_CACCT_CLONE8       GGGGCCGGGGGTGAGACCATGGGGAGCTGGGCTGGGGCTGGGGGTGAGACCATGGGGAGC    60
aWT_GGTTC_BAC           GGGGCCGGGGATGAGACCATGGGGAGCTGGG--------------------------------    0
aWT_GGTTC_CLONE5        GGGGCCGGGGATGAGACCATGGGGAGCTGGG--------------------------------    0
                        **********.********************

aDUP_CACCT_BAC          TGGGGCCGGGGGCTGGGACTAGTCCATGGGGAGCTGGGCTGGGGCTGGGGGTGAGACCAT    120
aDUP_CACCT_CLONE8       TGGGGCCGGGGGCTGGGACTAGTCCATGGGGAGCTGGGCTGGGGCTGGGGGTGAGACCAT    120
aWT_GGTTC_BAC           ------------------------------------------------------------    0
aWT_GGTTC_CLONE5        ------------------------------------------------------------    0


aDUP_CACCT_BAC          GGGGAGCTGGGCTGGGGCTGGGACTAGTCCATGGGGAGCTGGGCTGGAGCTGGGGGTGAG    180
aDUP_CACCT_CLONE8       GGGGAGCTGGGCTGGGGCTGGGACTAGTCCATGGGGAGCTGGGCTGGAGCTGGGGGTGAG    180
aWT_GGTTC_BAC           ------------------------------------------------------------    0
aWT_GGTTC_CLONE5        ------------------------------------------------------------    0


aDUP_CACCT_BAC          ACCATGGGGAGCTGGGGCCGGGGGCTGGGACTAGTCCATGGGGAGCTGGGCTGGGGCTGG    240
aDUP_CACCT_CLONE8       ACCATGGGGAGCTGGGGCCGGGGGCTGGGACTAGTCCATGGGGAGCTGGGCTGGGGCTGG    240
aWT_GGTTC_BAC           ------------------------------------------------------------    0
aWT_GGTTC_CLONE5        ------------------------------------------------------------    0


aDUP_CACCT_BAC          GGGTGAGACCATGGGGAGCTGGGCTGGGGCTGGGACTAGTCCATGGGGAGCTGGGGGTGA    300
aDUP_CACCT_CLONE8       GGGTGAGACCATGGGGAGCTGGGCTGGGGCTGGGACTAGTCCATGGGGAGCTGGGGGTGA    300
aWT_GGTTC_BAC           ------------------------------------------------------------    0
aWT_GGTTC_CLONE5        ------------------------------------------------------------    0


aDUP_CACCT_BAC          GACCATGGGGAGCTGGGGCCGGGGGCTGCGACTAGTCCATGGGGAGCTGGGCTGGGGCTG    360
aDUP_CACCT_CLONE8       GACCATGGGGAGCTGGGGCCGGGGGCTGCGACTAGTCCATGGGGAGCTGGGCTGGGGCTG    360
aWT_GGTTC_BAC           ------------------------------------------------------------    0
aWT_GGTTC_CLONE5        ------------------------------------------------------------    0


aDUP_CACCT_BAC          GGGGTGAGTCCATGGGGAGCTGGGCTGGGGCTGGGGGTGAGACCATGGGGAGCTGGGCTG    420
aDUP_CACCT_CLONE8       GGGGTGAGTCCATGGGGAGCTGGGCTGGGGCTGGGGGTGAGACCATGGGGAGCTGGGCTG    420
aWT_GGTTC_BAC           ------------------------------------------------------------    0
aWT_GGTTC_CLONE5        ------------------------------------------------------------    0


aDUP_CACCT_BAC          GGGCTGGGGGTGAGACCATGGGGAGCTGGGGCCGGGGGATGGGACTAGTCCATGGGGAGC    480
aDUP_CACCT_CLONE8       GGGCTGGGGGTGAGACCATGGGGAGCTGGGGCCGGGGGATGGGACTAGTCCATGGGGAGC    480
aWT_GGTTC_BAC           ------------------------------------------------------------    0
aWT_GGTTC_CLONE5        ------------------------------------------------------------    0


aDUP_CACCT_BAC          TGGGCTGGGGCTGGGGGTGAGACCATGGGGAGCTGGGCTGGGGCTGGGACTAGTCCATGG    540
aDUP_CACCT_CLONE8       TGGGCTGGGGCTGGGGGTGAGACCATGGGGAGCTGGG--------------------------    517
aWT_GGTTC_BAC           ------------------------------------------------------------    31
aWT_GGTTC_CLONE5        ------------------------------------------------------------    31


aDUP_CACCT_BAC          GGAGCTGGGCTGGAGCTGGGGGTGAGACCATGGGGAGCTGGGGCCGGGGGCTGCGACTAG    600
aDUP_CACCT_CLONE8       ------------------------------------------------------------    517
aWT_GGTTC_BAC           ----------------------------------------------G-------------    32
aWT_GGTTC_CLONE5        ----------------------------------------------G-------------    32


aDUP_CACCT_BAC          TCCATGGGGAGCTGGGCTGGGGCTGGGGGTGAGTCCATGGGGAGCTGGGCTGGGGCTGGG    660
aDUP_CACCT_CLONE8       ----------------------------------------------CTGGGGCTGGG    528
aWT_GGTTC_BAC           ----------------------------------------------CTGGGGCT-GG    42
aWT_GGTTC_CLONE5        ----------------------------------------------CTGGGGCT-GG    42
                                                                       ******* **

aDUP_CACCT_BAC          GGTGAGTCCATGGGGAGCTGGGGCTGGGGCTGGGACTAGTCCATGGGCAGCTGGGCTGGG    720
aDUP_CACCT_CLONE8       GGTGAGTCCATGGGGAGCTG----------------------------------------    548
aWT_GGTTC_BAC           GACTAGTCCATGGGGAGCTGG--------------------------------------    63
aWT_GGTTC_CLONE5        GACTAGTCCATGGGGAGCTGG--------------------------------------    63
                        *.  ****************

aDUP_CACCT_BAC          GCTGGGGGTGAGTCCATGGGGAGCTGGGCTGGGGCTGGGGGTGAGTCCATGGGGAGCTGG    780
aDUP_CACCT_CLONE8       ------------------------------------------------------------G    549
aWT_GGTTC_BAC           ------------------------------------------------------------G    64
aWT_GGTTC_CLONE5        ------------------------------------------------------------G    64
                                                                                     *

aDUP_CACCT_BAC          GCTGGGGTTGGGGGTGAGTCCATGGGGAGCTGGGCTGGGTCTCCTGGGGTTGCACCTGCA    840
aDUP_CACCT_CLONE8       GCTGGGGTTGGGGGTGAGTCCATGGGGAGCTGGGCTGGGTCTCCTGGGGTTGCACCTGCA    609
aWT_GGTTC_BAC           GCTGAAGTTGGGGGTGAGTCCATGGGGAGCTGGGCTGGGCCTCCTGGGGTTGCACCTGCA    124
aWT_GGTTC_CLONE5        GCTGAAGTTGGGGGTGAGTCCATGGGGAGCTGGGCTGGGCCTCCTGGGGTTGCACCTGCA    124
                        ****..********************************** ********************

aDUP_CACCT_BAC          CTCCTGTCTGCCCTTCCCTCTGCCGATGAAGCTCAGATCCCATGATAAGGAGGCACCTGC    900
aDUP_CACCT_CLONE8       CTCCTGTCTGCCCTTCCCTCTGCCGATGAAGCTCAGATCCCATGATAAGGAGGCACCTGC    669
aWT_GGTTC_BAC           CTCCTCTCTGCTCTTCCCTCTGCGTATGAAGCTCAGATCCCATGATAAGGAGGCACCTGC    184
aWT_GGTTC_CLONE5        CTCCTCTCTGCTCTTCCCTCTGCGTATGAAGCTCAGATCCCATGATAAGGAGGCACCTGC    184
                        ***** ***** ***********  ***********************************

aDUP_CACCT_BAC          AGACCAGGGGTCCTGCACGGACAGCCCCAGAGGTGGACATTGAGGACTCGTAGGAGGACT    960
aDUP_CACCT_CLONE8       AGACCAGGGGTCCTGCACGGACAGCCCCAGAGGTGGACATTGAGGACTCGTAGGAGGACT    729
aWT_GGTTC_BAC           AGACCAGGGGACCTGCACGGACAGCCCCAGAGGTGGACATTGAGGACTCGTAGGAGGACT    244
aWT_GGTTC_CLONE5        AGACCAGGGGACCTGCACGGACAGCCCCAGAGGTGGACATTGAGGACTCGTAGGAGGACT    244
                        **********:**************************************************

aDUP_CACCT_BAC          TGGGTCTCATACGGCGGGTGGGGAGCAGGGCCCCTTCCTGGCTGAGGACACTTGGTGCTG   1020
aDUP_CACCT_CLONE8       TGGGTCTCATACGGCGGGTGGGGAGCAGGGCCCCTTCCTGGCTGAGGACACTTGGTGCTG    789
aWT_GGTTC_BAC           TGGGTCTCATACGGCGGGTGGGGAGCAGGGCCCCTTCCTGGCTGAGGACACTTGGTGCTG    304
aWT_GGTTC_CLONE5        TGGGTCTCATACGGCGGGTGGGGAGCAGGGCCCCTTCCTGGCTGAGGACACTTGGTGCTG    304
                        ************************************************************

aDUP_CACCT_BAC          TCCCCTCTCAAGGCTGTTTCCCCATCTGACAAAGGGGTCTCATGTGAGCCTCCCACCAAG   1080
aDUP_CACCT_CLONE8       TCCCCTCTCAAGGCTGTTTCCCCATCTGACAAAGGGGTCTCATGTGAGCCTCCCACCAAG    849
aWT_GGTTC_BAC           TCCCCTCTCAAGGCTGTTTCCCCATCTGACAAAGGGGTCTCATGTGAGCCCCCAACCAAG    364
aWT_GGTTC_CLONE5        TCCCCTCTCAAGGCTGTTTCCCCATCTGACAAAGGGGTCTCATGTGAGCCCCCAACCAAG    364
                        ************************************************ **.******
```

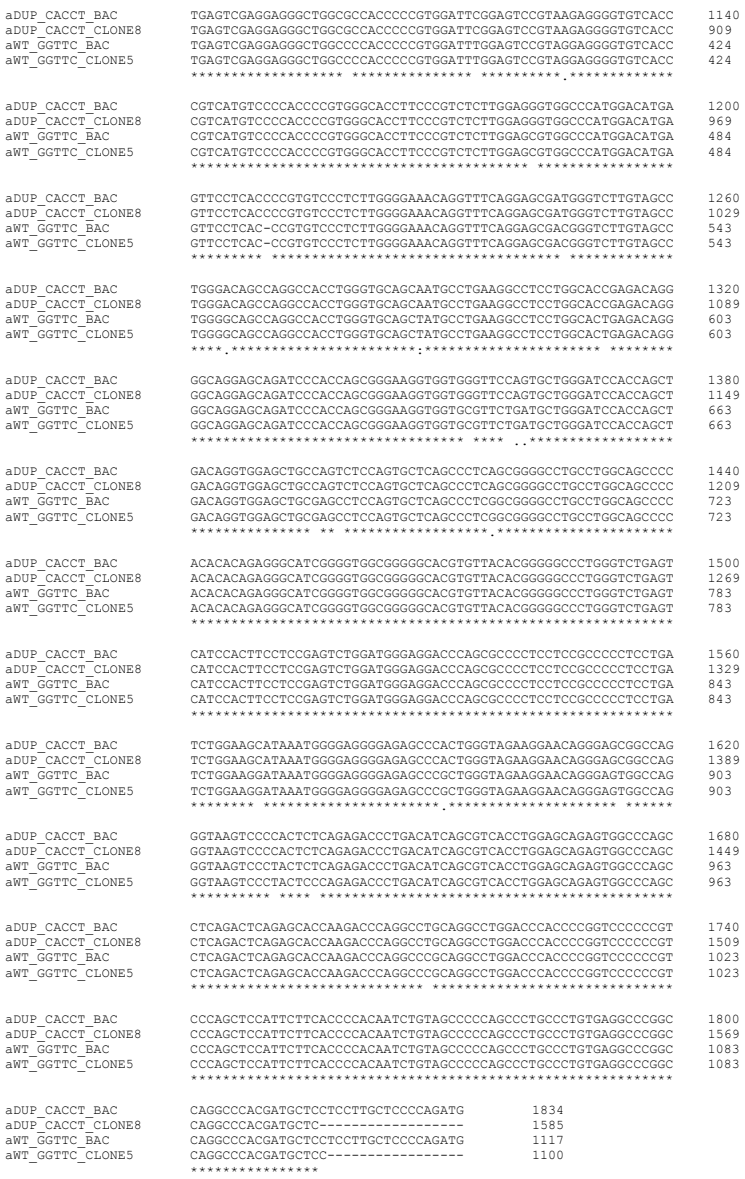
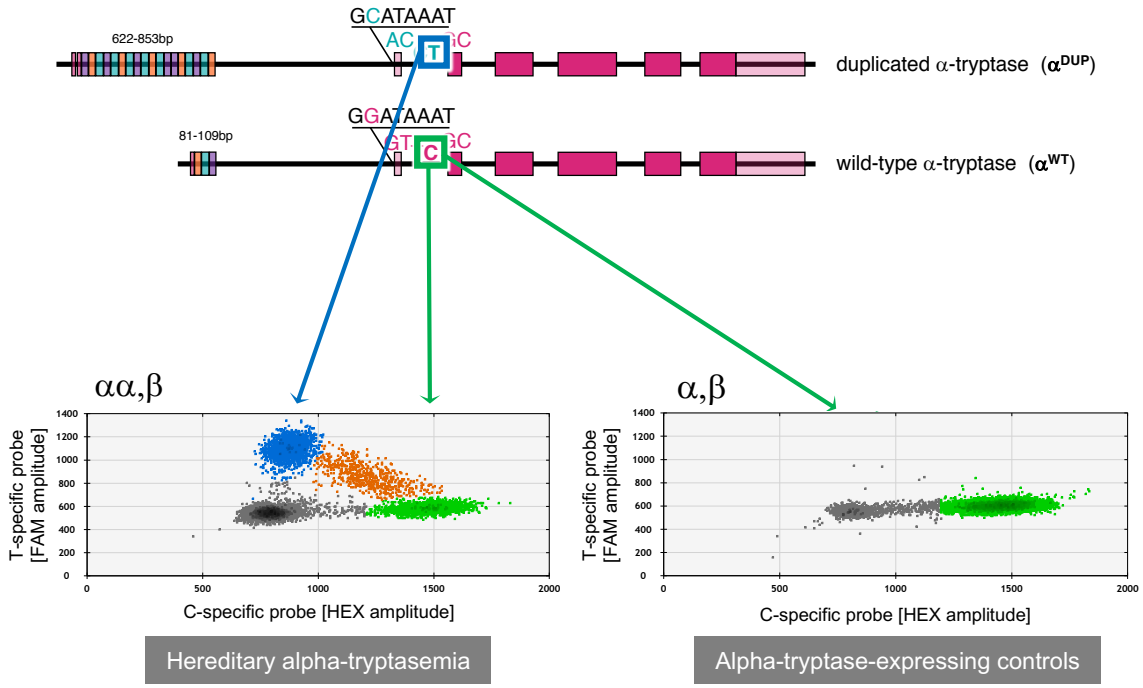


Figure S3. Multiple sequence alignment of representative cloned duplicated and wild-type α-tryptase promoters.



FWD: GGAGCAGAGTGGCCCAGCCTCAG
REV: ACGATGCCCACTCGCTGCAGGG (alpha-tryptase specific sequence in alpha^{WT} and alpha^{DUP})
PROBES: AGGCC**[C/T]**GCAGGCCTGGA

Figure S4. Alpha-tryptase isoform-specific promoter assay. (Top) Schematic of the two alpha-tryptase isoforms present at *TPSAB1* in a patient with hereditary alpha tryptasemia. In order to identify alleles containing the expanded promoter in linkage with duplicated alpha-tryptase (α^{DUP}) in other samples, a reverse primer was designed to hybridize only to alpha-tryptase sequences, while the sequence complementary to the forward primer were present at all tryptase isoforms. Two probes were then designed and multiplexed in order to compete with one another to detect the single base pair change C>T, with the latter being present 5' of the first coding exon of α^{DUP} (primer/probe sequences at bottom). When only α^{WT} was present, fluorescence was detected in only one channel (HEX) corresponding to the C-containing sequence (green, right middle). Whereas when α^{DUP} was present both a C-linked to α^{WT} as well as the C>T were detected (blue, FAM, left middle). Orange data-points are droplets containing both α^{WT} (C-specific, HEX) and α^{DUP} (T-specific, FAM) variants.

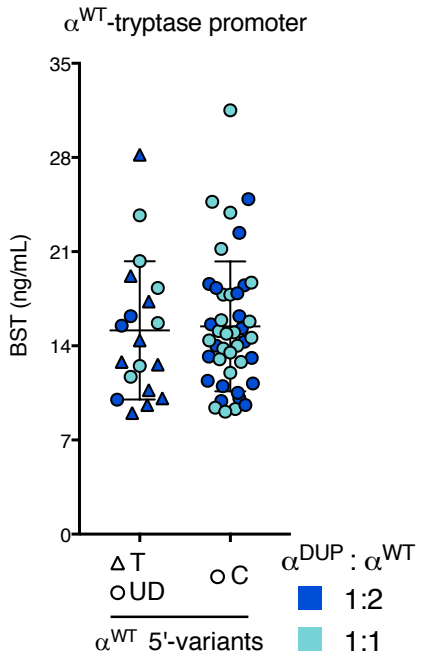


Figure S5. Unidentified and C>T associated wild-type alpha-tryptase promoters do not affect basal serum tryptase. Basal serum tryptase (BST) levels in patients with hereditary alpha tryptasemia separated by the promoter variant identified in linkage with wild-type alpha (α^{WT}) on the allele harboring a duplication. While all α^{WT} sequences on non-duplicated alleles are linked to the major allele, some α^{WT} sequences on alleles with increased *TPSAB1* copy number were not. Individuals with the common major allele C are shown on the right, and individuals with undefined (UD, circles) or the minor allele T (triangles) are shown on the left. The ratio of duplicated alpha-tryptase (α^{DUP}) to α^{WT} copy is indicated in blue (1:2) or cyan (1:1). Mann-Whitney.

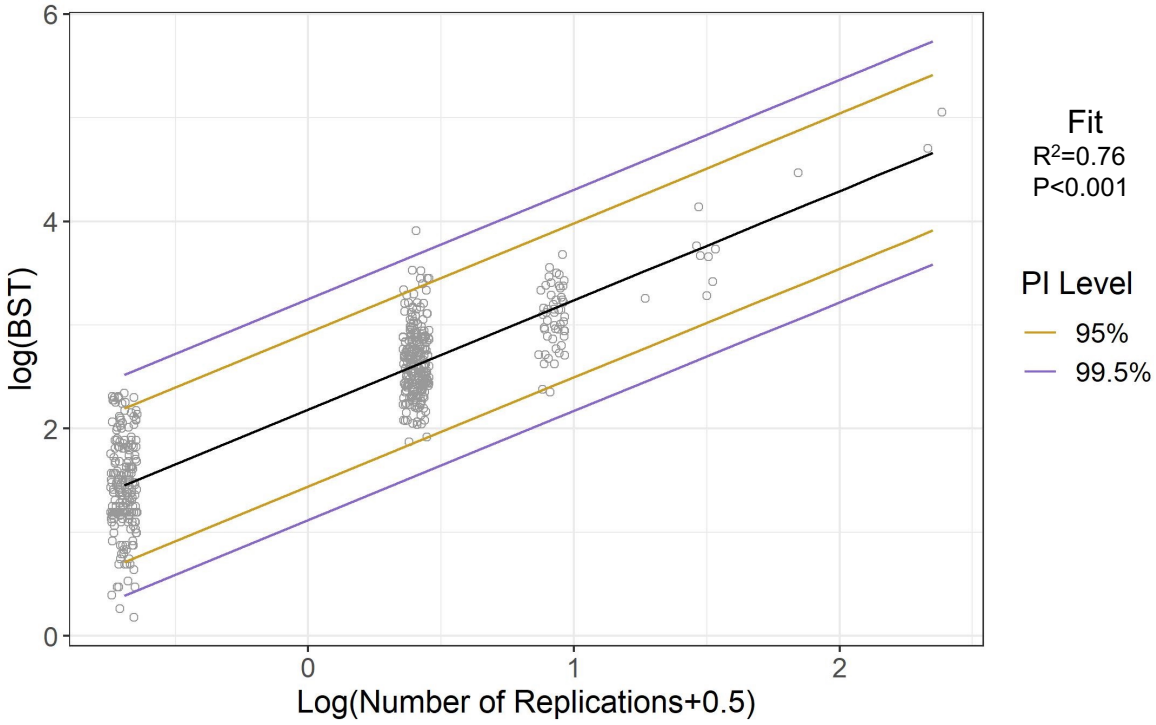


Figure S6. Log-transformed modeling of BST by *TPSAB1* copy number.

Table S1. Tryptase haplotype sequences.

CACCT	**C**ATAAATGGGGAGGGGAGAGCCC**A**CTGGGTAGAAGGAACA
	GGGAG**C**GGCCAGGGTAAGTCCC**C**ACTCTCAGAGACCCTGA
	CATCAGCGTCACCTGGAGCAGAGTGGCCCAGCCTCAGACT
	CAGAGCACCAAGACCCAGGCC**T**
GGTTC	**G**ATAAATGGGGAGGGGAGAGCCC**G**CTGGGTAGAAGGAACA
	GGGAG**T**GGCCAGGGTAAGTCCC**T**ACTCTCAGAGACCCTGA
	CATCAGCGTCACCTGGAGCAGAGTGGCCCAGCCTCAGACT
	CAGAGCACCAAGACCCAGGCC**C**
GACCC	**G**ATAAATGGGGAGGGGAGAGCCC**A**CTGGGTAGAAGGAACA
	GGGAG**C**GGCCAGGGTAAGTCCC**C**ACTCTCAGAGACCCTGA
	CATCAGCGTCACCTGGAGCAGAGTGGCCCAGCCTCAGACT
	CAGAGCACCAAGACCCAGGCC**C**
GACCT	**G**ATAAATGGGGAGGGGAGAGCCC**A**CTGGGTAGAAGGAACA
	GGGAG**C**GGCCAGGGTAAGTCCC**C**ACTCTCAGAGACCCTGA
	CATCAGCGTCACCTGGAGCAGAGTGGCCCAGCCTCAGACT
	CAGAGCACCAAGACCCAGGCC**T**

Table S2. 39-bp tryptase consensus sequences.

alpha^{WT}	CCC**G**CTGGGTAGAAGGAACAGGGAG**T**GGCCAGGATGCTGA**GC**
alpha^{DUP}	CCC**A**CTGGGTAGAAGGAACAGGGAG**C**GGCCAGGATGCTGA**GC**
beta	CCC**A**CTGGGTAGAAGGAACAGGGAG**C**GGCCAGGATGCTGA**AT**

blue – 5' UTR; black – first coding exon; red isoform-defining variants.

Table S3. Tryptase primer/probe sequences.

$alpha^{TOTAL}$	FWD: TGCTGCTGGCGCTGC REV: GACTCTCAGGCTCACCTGC PROBE: CTGCAGCAAGCGGGTATCGTC
$alpha^{WT}$	FWD: CCCGCTGGGTAGAAGGAAC REV: GACGATACCCGCTTGCTG PROBE: TGGCCAGGATGCTGAGC
$alpha^{DUP}$	FWD: CCCACTGGGTAGAAGGAAC REV: GACGATACCCGCTTGCTG PROBE: CGGCCAGGATGCTGAGC
beta	FWD: CCCACTGGGTAGAAGGAAC REV: AACGATGCCCACTCGCTG PROBE: CGGCCAGGATGCTGAAT

Table S4. Alpha-tryptase sequences and corresponding reference assemblies.

Position (relative to TSS)	-30	-8	36	44,45	177	309	333	508	644	647	657	661
Alpha1; M30038.1; CHM1; alpha^{WT}	G	T	G	GC	C	A	A	C	G	A	C	A
Alpha2; AF206665.1; BC051852.1; BC028059.1; LT733899.1; KJ892311.1; DQ894235.2; AY893004.1	G	T	G	CG	T	G	A	C	C	G	A	C
AC238650.2; AF098328.1; AF206666.1; BC028059.1; BC051852.1; GM243385; NA12878	G	T	C	CG	T	G	A	C	C	G	A	C
FJ931116.1	G	T	G	CG	C	G	C	T	G	A	C	A
AC240106.3	G	T	C	CG	T	G	A	C	G	A	C	A
alpha^{DUP}	**A**	**C**	G	GC	C	A	A	C	G	A	C	A

>Alpha1
cccgctgggtagaaggaacagggagtggccaggATGCTGAGCCTGCTGCTGCTGGCGCTGCCCGTCCTGGCGAGCCGCGCC
TACGCGGCCCCTGCCCCAGTCCAGGCCCTGCAGCAAGCGGGTATCGTCGGGGGTCAGGAGGCCCCCAGGAGCAAGTGGCCC
TGGCAGGTGAGCCTGAGAGTCCGCGACCGATACTGGATGCACTTCTGCGGGGGGCTCCCTCATCCACCCCCAGTGGGTGCTG
ACCGCGGCGCACTGCCTGGGACCGGACGTCAAGGATCTGGCCACCCTCAGGGTGCAACTGCGGGAGCAGCACCTCTACTAC
CAGGACCAGCTGCTGCCAGTCAGCAGGATCATCGTGCACCCACAGTTCTACATCATCCAGACTGGAGCGGATATCGCCCTG
CTGGAGCTGGAGGAGCCCGTGAACATCTCCAGCCGCGTCCACACGGTCATGCTGCCCCCTGCCTCGGAGACCTTCCCCCCG
GGGATGCCGTGCTGGGTCACTGGCTGGGGCGATGTGGACAATGATGAGCCCCTCCCACCGCCATTTCCCCTGAAGCAGGTG
AAGGTCCCCATAATGGAAAACCACATTTGTGACGCAAAATACCACCTTGGCGCCTACACGGGAGACGACGTCCGCATCATC
CGTGACGACATGCTGTGTGCCGGGAACAGCCAGAGGGACTCCTGCAAGGGCGACTCTGGAGGGCCCCTGGTGTGCAAGGTG
AATGGCACCTGGCTACAGGCGGGCGTGGTCAGCTGGGACGAGGGCTGTGCCCAGCCCAACCGGCCTGGCATCTACACCCGT
GTCACCTACTACTTGGACTGGATCCACCACTATGTCCCCAAAAAGCCGTGA

Table S5. Percentage of healthy individuals expressing specific α- and β-tryptase isoforms.

Transcript	Haplotype	Sample number (n = 4160)	% Total	% Tryptase positive
any tryptase	-	863	20.7%	100%
any α	ACAT	741	17.8%	85.9%
any β	NNGC	314	7.5%	36.4%
α^{DUP}	**ACGC**	**25**	**0.6%**	**2.9%**
α^{WT}	GTGC	289	7.2%	33.5%

Data are from combined from 58 publicly available datasets; red – prevalence of hereditary alpha-tryptasemia

Table S6. Candidate variants of undetermined significance identified in patients with BST >11.4ng/mL who did not have HαT.

Patient	Gene	Chr	Start	End	Ref	Var	AA change	PBMC	BMGran	BMMC
1	*TET2*	4	105234636	105234636	C	T	p.Q232X	0.018923	0.2238426	0.1432696
1*	*FADS6*	17	74893497	74893497	C	CGGTTCCATG GGCTCCGTA	p.P33fs7X	0.027044	0.1444073	0.1624679
1	*GRB14*	2	164508777	164508777	G	GT	p.H297fs1X	0	0.18850502	0.04259754
2	*KMT2C*	7	152247975	152247975	G	A	p.T820I	0.05543634	0.0711392	0.22914893
2*	*FADS6*	17	74893497	74893497	C	CGGTTCCATG GGCTCCGTA	p.P33fs7X	0	0.1610098	0.08378218
2	*KMT5A*	12	123390764	123390764	C	T	p.R49W	0.02342595	0.06664452	0.26906613
2	*MSH3*	5	80654896	80654896	G	C	p.A57P	0.07519636	0.1615971	0.62264769
3	*MAP2K1*	15	66491063	66491063	A	C	3'UTR	0	0.16156567	0.06649085
3	*MAP2K1*	15	66491059	66491059	G	C	3'UTR	0	0.16472012	0.07177007
3	*MAP2K1*	15	66491055	66491055	G	T	3'UTR	0	0.16472012	0.05015322
4	*NOTCH2*	1	120069404	120069404	C	T	p.M1?	0.14917127	0.20416667	0.20916334
4	*RPL10*	X	154400837	154400837	C	T	p.R210W	0	0.14081633	0.14257028
5†	*RUNX1*	21	34792308	34792308	A	G	p.S424P	0.26548673	0.19277108	0.17142857
5†	*CEBPA*	19	33301848	33301848	GGGC	G	p.P189del	0.09615385	0.01497006	0. 02076125

Chr - chromosome; Ref - reference; Var - variant; AA - amino acid; PBMC - peripheral blood mononuclear cell; BMGran - bone marrow granulocytes; BMMC - bone marrow mononuclear cells; *indicated a shared insertion present in patient 1 and 3; †identified variants were germline; patient 4 had idiopathic hypereosinophilic syndrome.