Describing variants

"mutation nomenclature"

recommendations for the description of DNA changes

Johan den Dunnen

Human Genome Variation Society (HGVS)

VarNomen @ HGVS.org

http://www.HGVS.org/mutnomen/

(HUGO-MDI initiative)

Human and Clinical Genetics

HGVS / HVP / HUGO Sequence Variant Description working group

Working Group Members:

- Anne-Francoise Roux (EGT)
- Donna Maglott (NCBI/EBI)
- Jean McGowan-Jordan (ISCN)
- Peter Taschner (LSDBs)
- Raymond Dalgleish (LSDBs)
- Reece Hart (industry)
- Johan den Dunnen (chair)
- HGVS - Marc Greenblatt
- HUGO - Stylianos Antonarakis

Nomenclature

(describing DNA variants)

Stable

Meaningful ***Memorable***

Unequivocal

Definitions

- **prevent confusion**
  - ***mutation***
    - *change*
    - *disease-causing change*
  - ***polymorphism***
    - *change in >1% population*
    - *not disease causing change*

- **better use neutral terms**
  - ***sequence variant***
  - ***allelic variant***
  - ***alteration***
  - ***CNV*** *(Copy Number Variant)*
  - ***SNV*** *(not SNP)*

Variant description

the basis

HUMAN MUTATION 15:7–12 (2000)

MDI SPECIAL ARTICLE

Mutation Nomenclature Extensions and Suggestions to Describe Complex Mutations: A Discussion

Johan T. den Dunnen[1*] and Stylianos E. Antonarakis[2*]
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Consistent gene mutation nomenclature is essential for efficient and accurate reporting, testing, and curation of the growing number of disease mutations and useful polymorphisms being discovered in the human genome. While a codified mutation nomenclature system for simple DNA lesions has now been adopted broadly by the medical genetics community, it is inherently difficult to represent complex mutations in a unified manner. In this article, suggestions are presented for reporting just such complex mutations. Hum Mutat 15:7–12, 2000.

KEY WORDS: complex mutation; mutation detection; mutation database; nomenclature; MDI

HGVS
HUMAN GENOME VARIATION SOCIETY

http:// www.HGVS.org / mutnomen

on behalf of HUGO MDI / HGVS

www.HGVS.org/mutnomen

HGVS HUMAN GENOME VARIATION SOCIETY

Nomenclature for the description of sequence variants

(last modified March, 2014)

Prepared by Johan den Dunnen

Google Search www.HGVS.org

Google Search www.facebook.com/

Follow the recommendations
when you disagree, ***start a debate*** *-*
do not use private rules, this only causes confusion

Contents

Questions ?

- mail to "*VarNomen @ HGVS.org*"

Recent additions

- NEW Proposals open for comments
- follow HGVS on Facebook
- Proposal for description translocations *(presented at HGVS2013, Peter Tas*
- RNA editing
- proposal for complex variants
  (published: Peter Taschner et al., Human Mutation 32:507-511)

Current recommendations

- Introduction
- General recommendations
- Versioning
  - HGVS versioning *(all versions explained)*
  - Version list *(changes after V2.0)*
- Use a Locus Reference Genomic sequence (LRG)
- Specific recommendations
  - DNA-level
  - RNA-level
  - Protein-level

Background material

- Nucleotide numbering
- Standards *(definitions, symbols, nucleotide*
- NEW The basics - slide presentation (
- Checklist *(online help when writing publicat...)*

Example descriptions

- DNA
- RNA
- Protein
- Quick Reference *(simple examples)*

Discussions

- General
- Reference sequence
- Nucleotide numbering

FAQ (frequently asked questions)

LUMC *Human and Clinical Genetics*

facebook & twitter

facebook

HGVS
Education

Timeline | About | Photos | Likes | Events

PEOPLE

217 likes

ABOUT

These HGVS pages will be used to discuss any subject we encounter regarding the "Recommendations for the description of sequence variants".

http://www.HGVS.org/mutnomen

PHOTOS

HGVS shared a link.
October 19

Tue. Oct. 21, 12:30-14:00, HVP Sequence Variant Description workshop ASHG, room 28A, San Diego Convention Center. What are we going to do? Discuss variant nomenclature!
After a short introduction on the basics, the
open... See More

Schedule of Events | AS
www.ashg.org
The American Society of H
Incorporated | 9650 Rock
Maryland 20814society@a
• (301) 634-7300Privacy

HGVS
October 17

Intron after stop codon
Q: how do I number a variant which is at position 13 in an intron immediately following the last nucleotide (c.876) of the stop codon? c.*0+13C>T can not be since HGVS does not use position "0".
A: since the variant is in an intron at position 13 after nucleotide c.876 the correct description is c.876+13C>T.
Interesting to note is that in this peculiar example nucleotides in the intron are numbered like c.876+1, c.876+2, c.876+3, … c.*1-3, c.*1-2, c.*1-1.

JT den Dunnen @jtdendunnen 6d
HGVS and ISCN
HGVS made recommendations to describe variants at nucleotide level. However, first variants... fb.me/2xWBGUDly

JT den Dunnen @jtdendunnen 11 Oct
Unique indel being an inversion
Q: how to describe variant c.3821_3825delTCACTinsAGTGA, an in-frame indel... fb.me/1hJnxny03

JT den Dunnen @jtdendunnen 11 Oct
The basiscs - slide presentation .. now updated.
The slide presentation explaining the basics of the variant... fb.me/2778rhFVz

Versioning

Why versioning?

Last modified May, 2010

Versioning

The recommendations for the description of sequence variants are designed to be ***stable***, ***meaningful***, ***memorable*** and ***unequivocal***. Still, every now and then small modifications will need to be made to remove small inconsistencies and/or to clarify confusing conventions. In addition, the recommendations may be extended to resolve cases that were hitherto not covered. To allow users to specify up to what point they follow the HGVS recommendations we will start to work with version numbers. As of now, ***any change*** in the recommendations will get a new, ***incremental version number***. All changes introduced in a new version will be specified on the version list.

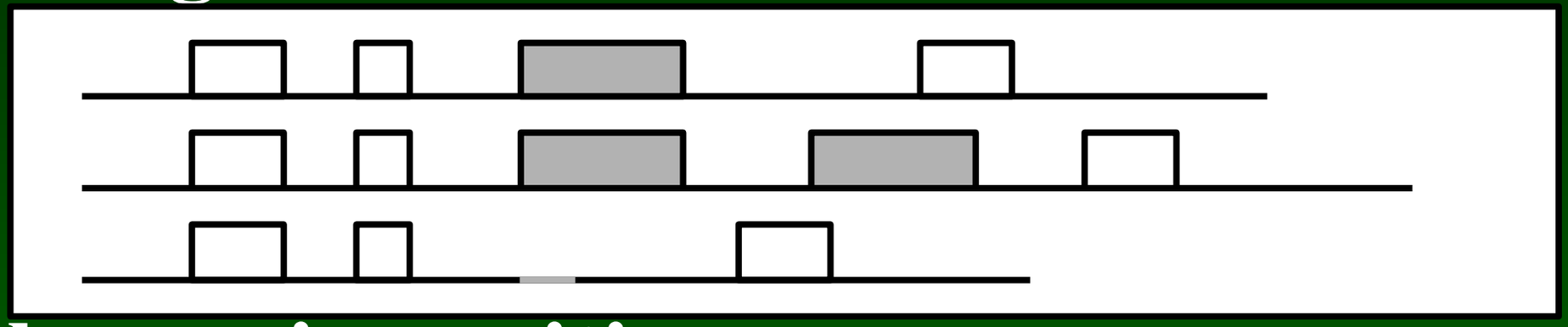
The recommendations described in the upcoming publication will be known as the ***HGVS recommendations for the description of sequence variants*** - version 2.0.

Variant types

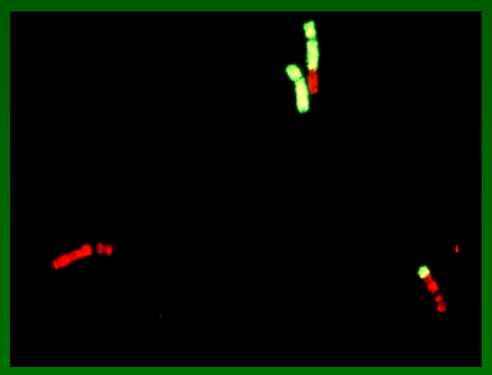
- **change in sequence**

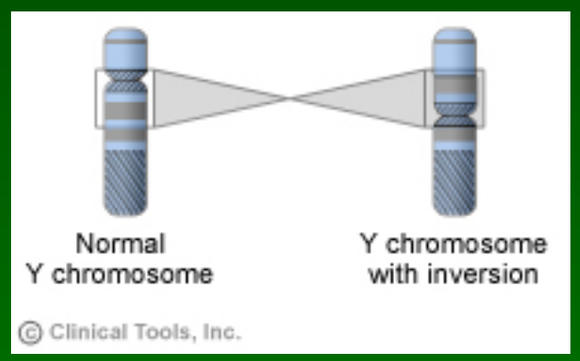
```
ACATCAGGAGAAGATGTTC GAGACTTTGCCA
ACATCAGGAGAAGATGTTT GAGACTTTGCCA
ACATCAGGAGAAGATGTT  GAGACTTTGCCA
ACATCAGGAGAAGATGTTCCGAGACTTTGCCA
```

- **change in amount** *(Copy Number Variation)*



- **change in position**





Structural Variation (SV)

DNA, RNA, protein

- **unique descriptions**
  prevent confusion

- **DNA**
  A, G, C, T
  g.957A>T, c.63-3T>C

- **RNA**
  a, g, c, u
  r.957a>u, r.(?), r.spl?

- **protein** *(mostly deduced)*
  three / one letter amino acid code
  ** = stop codon*
  p.(His78Gln)

Reference sequence

- **use official HGNC gene symbols**

- **provide reference sequence**
  - ***covering complete sequence***
    - ***largest transcript***
  - ***preferably a LRG***
    - ***e.g. LRG_123***
  - ***give accession.version number***
    - ***e.g. NM_012654.3***

- **indicate type of Reference Sequence**
  - ***DNA***
    - ***coding DNA c.***
    - ***genomic g.***
    - ***mitochondrial m.***
    - ***non-coding RNA n.***
  - ***RNA r.***
  - ***protein p.***

The LRG

Dalgleish *et al. Genome Medicine* 2010, 2:24
http://genomemedicine.com/content/2/4/24

Genome Medicine

CORRESPONDENCE Open Access

Locus Reference Genomic sequences: an improved basis for describing human DNA variants

Raymond Dalgleish[1]*, Paul Flicek[2], Fiona Cunningham[2], Alex Astashyn[3], Raymond E Tully[3], Glenn Proctor[2], Yuan Chen[2], William M McLaren[2], Pontus Larsson[2], Brendan W Vaughan[2], Christophe Béroud[4], Glen Dobson[5], Heikki Lehväslaiho[6], Peter EM Taschner[7], Johan T den Dunnen[7], Andrew Devereau[5], Ewan Birney[2], Anthony J Brookes[1] and Donna R Maglott[3]

Abstract

As our knowledge of the complexity of gene architecture grows, and we increase our understanding of the subtleties of gene expression, the process of accurately describing disease-causing gene variants has become increasingly problematic. In part, this is due to current reference DNA sequence formats that do not fully meet present needs. Here we present the

Introduction

In 1993 Ernest Beutler
editor of the *American*
lighting the deficienci
describe DNA variants
Human Mutation invit
Tsui to produce a nome
proteins [2]. From thes
years have borne witn

EDITORIAL

nature genetics

Conventional wisdom

Recent agreement on stable reference sequences for reporting human genetic variants now allows us to mandate the use of the allele naming conventions developed by the Human Genome Variation Society.

By agreement between stakeholders and two principal databases, it has been proposed (R. Dalgleish *et al.*, *Genome Med.* 2, 24, 2010, doi:10.1186/gm145) that human genetic variants be reported relative to a new set of stable reference sequences, "Locus Reference, Genomic" (LRG, pronounced "large" http://www.lrg-sequence.org/page.php). These sequences have been developed from the initial NCBI RefSeqGene concept and are provided by NCBI and EBI according to agreed rules

age, resequencing and marker association studies and so keep allele descriptions commensurate with the method by which their data were generated.

The LRG reference sequences should be used in conjunction with standard HGNC gene abbreviations (http://www.genenames.org/) that we already require as a condition of publication. All human genetic variants must now be described—in abstracts and at first use—in accor-

EBI, NCBI, Gen2Phen

GEN2PHEN

LUMC *Human and Clinical Genetics*

© JT den Dunnen

Numbering residues

- **start with 1**
  - *genomic* *1 is first nucleotide of file*
    - *no +, - or other signs*
  - *coding DNA* *1 is A of ATG*
    - *for introns refer to genomic Reference Sequence*

- **repeated segments** *(…CGTGTG TG A…)*
  - *assume most 3' as changed*

- **coding DNA only**
  - *5' of ATG …, -3, -2, -1, A, T, G, …*
    - *no nucleotide 0*
  - *3' of stop *1, *2, *3, …*
    - *no nucleotide 0*
  - *intron*
    - *position between nt's 654 and 655*
    - *c.654+1, +2, +3, ……., -3, -2, c.655-1*
    - *change + to - in middle*

Numbering

- **RNA** *(deduced mostly)*

like coding DNA

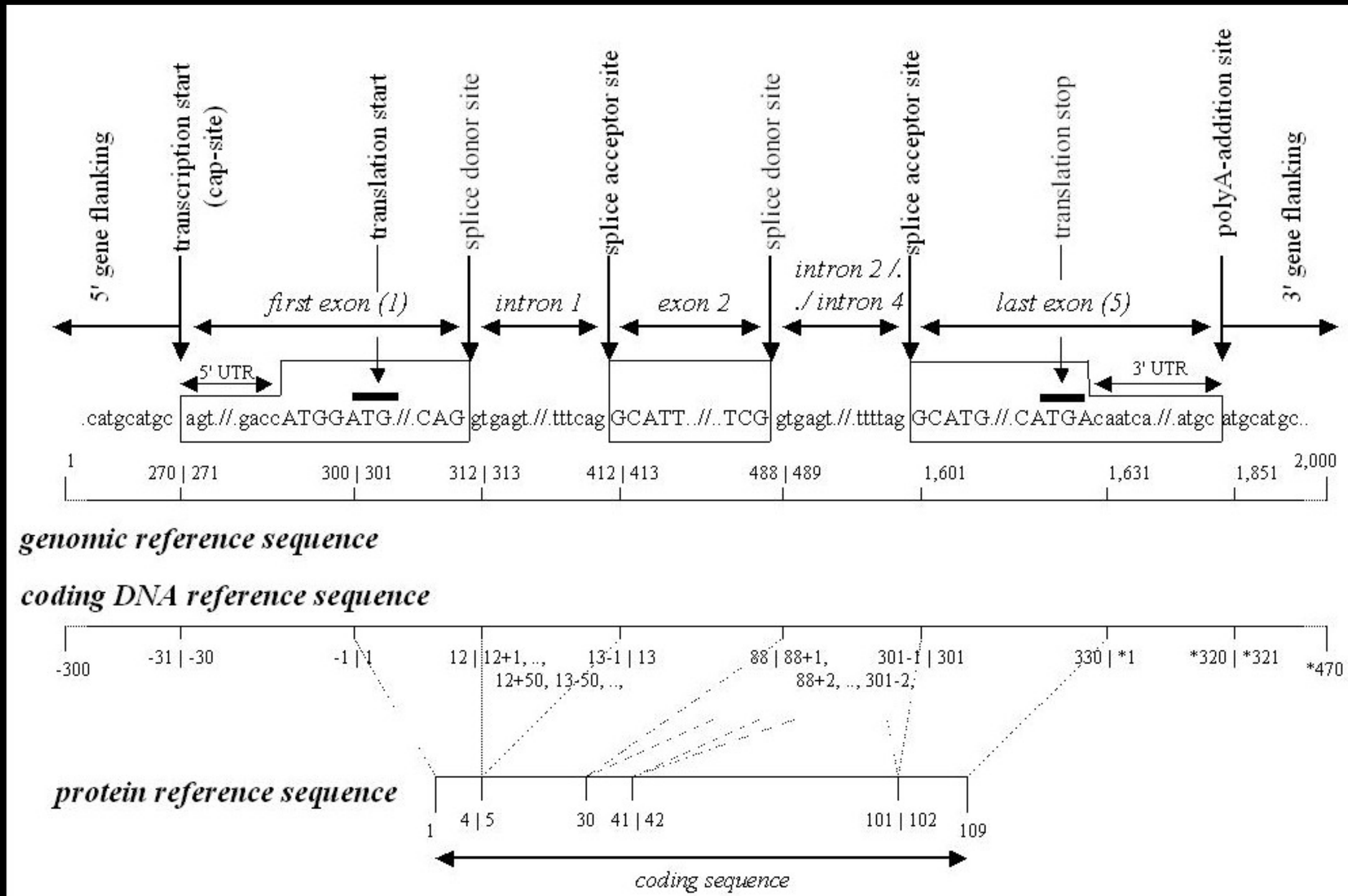
- **protein** *(deduced only)*

from first to last amino acid

rule of thumb: c. nucleotide position divided by 3 roughly gives amino acid residue

description between parantheses

Reference Sequence



coding DNA or genomic ?

- **human genome sequence**
  - *complete*
  - *covers all transcripts*
    - *different promoters, splice variants, diff. polyA-addition, etc.*
  - *but*
    - *hg19 chr2:g.121895321_121895325del*
    - *is long & complicated*
    - *huge reference sequence files*
    - *new builds follow each other regularly*
    - *carries no understandable information*

- **coding DNA**
  - *does not cover all variants*
  - *but gives a clue towards position*

Numbering - genomic

- g.63A>G
- g.859>C
- g.2396G>A
- g.5623C>G
- g.9443A>T
- g.12333T>G
- g.18979G>C

no relation to RNA & protein

Numbering - coding DNA

- **c.1637A>G**
  protein coding region
- **c.859+12T>C**
  in intron (5' half)
- **c.2396-6G>A**
  in intron (3' half)

relation to RNA & protein

- **c.-23C>G**
  5' of protein coding region (5' of ATG)
- **c.*143A>T**
  3' of protein coding region (3' of stop)
- **c.-89-12T>G**
  intron in 5' UTR (5' of ATG)
- **c.-649+79G>C**
  intron in 3' UTR (3' of stop)

HGVS HUMAN GENOME VARIATION SOCIETY

Types of variation

- **simple**
  - *substitution* — *c.123A>G*
  - *deletion* — *c.123delA*
  - *duplication* — *c.123dupA*
  - *insertion* — *c.123_124insC*
  - *other*
    conversion, inversion, translocation, transposition

- **complex**
  - *indel* — *c.123delinsGTAT*

- **combination of variants**
  - *two alleles* — *c.[123A>G];[456C>T]*
  - *>1 per allele* — *c.[123A>G;456C>T]*

Substitution

- **substitution designated by ">"**
  - ***> not used on protein level***

- **examples**

genomic	*g.54786A>T*
cDNA	*c.545A>T*
	(NM_012654.3 : c.546A>T)
RNA	*r.545a>u*
protein	*p.(Gln182Leu)*

Deletion

- **deletion**
  designated by "del"
  range indicated by "_"
- **examples**

c.546del
c.546delT

c.586_591del
c.586_591delTGGTCA, NOT c.586_591del6

c.(780+1_781-1)_(1392+1_1393-1)del
exon 3 to 6 deletion, breakpoint not sequenced

Duplication

- **duplication**
  designated by "dup"
  range indicated by "_"

- **examples**

c.546dup
c.546dupT

c.586_591dup
c.586_591dupTGGTCA, NOT c.586_591dup6
do not describe as insertion

c.(780+1_781-1)_(1392+1_1393-1)dup
exon 3 to 6 duplication, breakpoint not sequenced
NOTE: dup should be in tandem

Insertion

- **insertion**
  designated by "ins"
  range indicated by "_"
  ! give inserted sequence

- **examples**

c.546_547insT
NOT c.546insT or c.547insT

c.1086_1087insGCGTGA
NOT c.1086_1087ins6

c.1086_1087insAB567429.2:g.34_12567
when large insert submit to database and
give database accession.version number

Inversion

- **inversion**
  - *affecting at least 2 nucleotides*
  - *designated by "inv"*
  - *range indicated by "_"*

- **example**

  c.546_2031inv

  NOT c.2031_546inv

Conversion

- **conversion**
  - ***affecting at least 2 nucleotides***
  - ***designated by "con"***
  - ***range indicated by "_"***

- **examples**
  - ***c.546_657con917_1028***
  - ***c.546_2031conNM_023541.2:c.549_2034***

Sequence repeats

- **mono-nucleotide stretches**
  g.8932A(18_23)

  c.345+28T(18_23)
  alleles 345+28T[18];[21]

() = uncertain

- **di-nucleotide stretches**
  c.1849+363CAG(13_19)
  c.1849+363_1849+365(13_19)

- **larger**
  g.532_3886(20_45)
  3.3 Kb repeat

SNVs *(SNPs)*

- **SNV's**

***at least once** give description based on genome reference sequence*

hg19 chr9:g.3901666T>C

rs12345678:T>C

dbSNP entry

Characters & codes

- **codes used**

+, -, *	
>	***substitution (nucleotide)***
_	***range***
;	***separate changes (in/between alleles)***
,	***more transcripts***
( )	***uncertain***
[ ]	***allele***
=	***equals reference sequence***
?	***unknown***
del	***deletion***
dup	***duplication***
ins	***insertion***
inv	***inversion***
con	***conversion***
ext	***extension***
fs	***frame shift***

Uncertainty breakpoints

- **Copy Number Variants**

(last-normal_first-changed) _ (last-changed_first-normal) del

BAC / PAC probe

(AC123322.10_AL109609.5)_(AL451144.5_AL050305.9)del
chrX:g.(32,218,983_32,238,146)_(32,984,039_33,252,615)del
NCBI build 36.1

SNP-array

(rs2342234_rs3929856)_(rs10507342_rs947283)del
chrX:g.(32,218,983_32,238,146)_(32,984,039_33,252,615)del
GRCh36.p2

Uncertainty breakpoints 2

- **whole exon changes**

c.(423+1_424-1)_(631+1_632-1)del
intragenic deletion

c.(?_-79)_(631+1_632-1)del
deletion incl. 5' end

c.(423+1_424-1)_(*763_?)del
deletion incl. 3' end

c.(?_-79)_(*763_?)del
whole gene deletion, start/end undefined

describe what was actually tested

Alleles

- **allele**
  indicated by "[ ]", separated by ";"
- **2 changes, 2 alleles**
  c. [428A>G] ; [83dupG]
- **1 allele, several changes**
  c. [12C>G ; 428A>G ; 983dupG]
- **2 changes, allele unknown**
  c. [428A>G (;) 83dupG]
- **special cases**
  mosaicism
  c. 428A= / A>G
  chimerism
  c. 428A= // A>G

spaces in description used for clarity only

Complex

- **deletion / insertions**

  "indel"

  c.1166_1177delinsAGT

- **descriptions may become complex**

  when only an expert understands the "code" consider database submission

  description: c.875_941delinsAC111747.1

Changes in RNA

- **description like DNA**

  r. / a, g, c, u

- **examples**

r.283c>u
r.0 no RNA from allele
r.? effect unknown
r.spl affects RNA splicing
r.(spl?) may affect splicing
r.= no change
(equals reference sequence)

r.[=, 436_456del]
two transcripts from 1 allele

Changes in RNA$_2$

- **one allele, 2 transcripts**
  effect on splicing not 100%

c.456+3G>C

on RNA r.[=, 436_456del]

> p.[=, Arg146_Lys152del]

Changes in protein

- **description like DNA**

  p. / Ala, Cys, Gly, His, …, Ter
  p. / A, C, D, E, F, G, H, …, *

- **examples**

nonsense *no RNA data*
p.Trp65* ***(p.W65* / p.Trp65Ter)*** *r.(?)* *p.(Trp56*)*
no stop
p.*1054Glnext*31
p.0 - no protein
p.Met1? - likely, but unknown effect
NOT p.Met1Val
fs - frame shift change

Frame shifts

- **short form** *(sufficient)*

  p.Arg83fs

- **long from** *(more detail)*

p.(Arg83Serfs*15) *(no RNA analysis)*

indicate

first amino acid changed
position
first changed amino acid
length shifted frame
(from first changed to * incl.)
do not describe del, dup, ins, etc.

do not try to include changes at DNA level

Recent additions

- **added versioning**
  - *to support users*
    - *easier to find latest changes*
    - *allows statement "following HGVS version 2.0"*
- **stricter definitions**
  - *separate different classes*
  - *added hierarchy*
    - *computer-generated description*
    - *automated error-checking (Mutalyzer)*
- **simplified use special characters**
  - *" ", ";", "+", "*", …*
  - *improved consistency*

Complex changes$_2$

Taschner 2011. Hum.Mutat., 32: 507.

- **SUGGESTED: nested descriptions**

simplified description complex changes

g. 100_200inv {158A>C}

change

difference with original