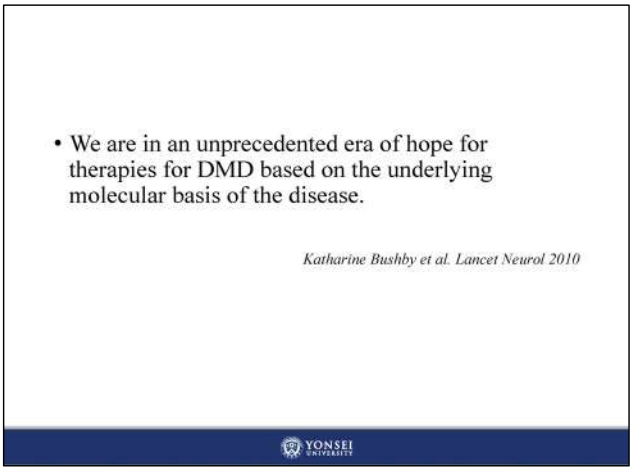
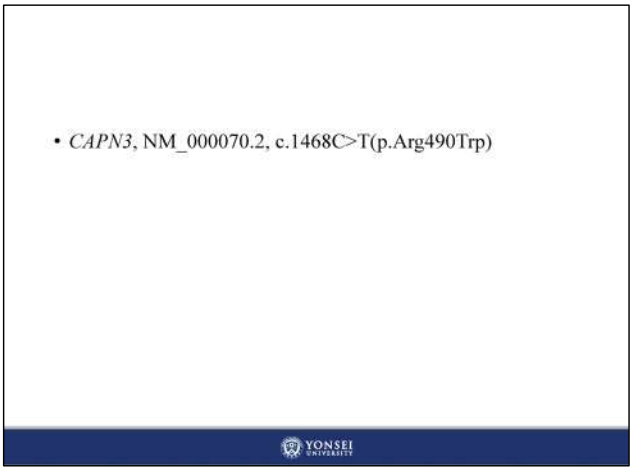
신경계질환에서 유전체 분석

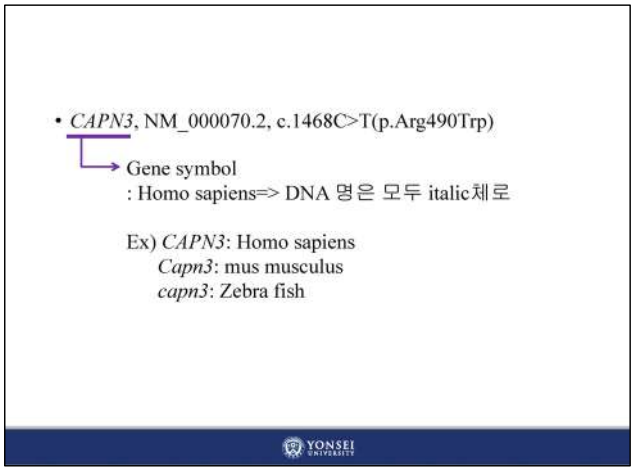
박 형 준
연세의대

Genomic analysis in neurologic diseases

Hyung Jun Park
Department of Neurology, Gangnam Severance Hospital, Yonsei University College of Medicine







• *CAPN3*, NM_000070.2, c.1468C>T(p.Arg490Trp)

→ Reference sequence
: 여러 개의 transcript중 대표적인 sequence
⇒ 일반적으로 가장 큰 transcript를 기준
⇒ 많은 경우 transcript 1이나 아닌 경우도 많음.
⇒ 각각 유전자에 대한 DATABASE에서 확인가능

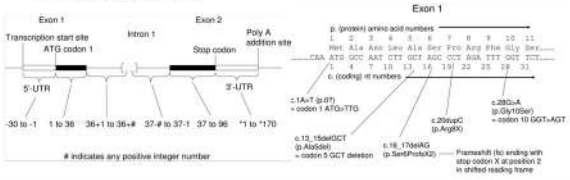
GNE myopathy: new nam

New Nomenclature[1]			Previous Nomenclature				
hGNE2 isoform	mRNA transcript	Exon	hGNE1 isoform	mRNA transcript	Exon	Ethnicity[2]
GenBank hGNE2 AP_001121699	GenBank Variant 1 NM_001128227	GenBank GNE gDNA NC_000009	GenBank hGNE1 NP_005467	GenBank Variant 2 NM_005476		
ENSEMBL GNE-003 ENSP 00000379859	ENSEMBL GNE-003 ENST 00000396594	ENSEMBL GNE gDNA ENSG 00000119125	ENSEMBL GNE-001 ENSP 00000357234	ENSEMBL GNE-001 ENST 00000377902		
p.C44S	c.131G>C	3	p.C33S	c.98G>C	2	Japanese
p.D207V	c.620A>T	4	p.D150V	c.527A>T	3	Japanese
p.V403L	c.1207G>C	11	p.V337L	c.1174G>C	10	Japanese
p.R1R1	c.1853T>C	11	p.I387T	c.1760T>C	10	Cajun, Roma Gypsies
p.Y727M	c.2179G>A	13	p.V690M	c.2070G>A	12	Indian
p.M745T	c.2235T>C	13	p.M722T	c.2135T>C	12	Middle Eastern

[1]Department of Neurology, Hadassah

• *CAPN3*, NM_000070.2, c.1468C>T(p.Arg490Trp)

Start codon으로부터 exon의 염기서열순서를 표기
- c.1468C>T : start condon ATG의 A를 1로 할 때 1468번째 염기가 원래 C(cytosine) 인데 T(thymine) 로 치환
- p.Arg490Trp: start condon Met를 1로 할 때 490째 아미노산이 원래 Arg(arginine) 인데 T(tryptophan)로 치환



Genomic analysis

ALD, ABCD1 gene

[Result]

상기환자의 ABCD1 (ATP-binding cassette, sub-family D, member 1) gene에 대한 염기서열 분석결과 돌연변이가 검출되지 않았습니다.

• Human Genome Mutation Database (http://www.hgmd.org)

Gene	Exon	Nucleotide change	Amino acid change	Mutation Effect
ABCD1	-	-	-	-

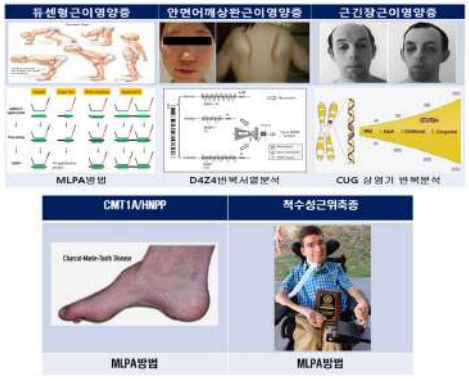
[Test Information]

1. Specimen: Genomic DNA isolated from peripheral blood leukocyte
2. Target gene: ABCD1
3. Chromosomal Locus: Xq28
4. OMIM : #300100 for disease : *300371 for gene
GeneBank accession number : NT_167198.1 : NM_000033.3

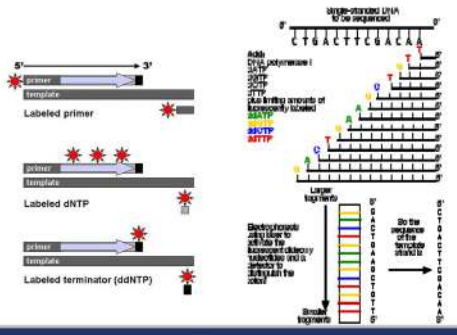
본 검사는 PCR-Sequencing방법으로 ALD환자의 약 6% 정도에서는 한계이상 Exon의 duplication 이나 deletion에 의해 유발되는데 본 검사법(PCR-Sequencing법)으로는 검출이 불가능하므로 임상적으로 의심되시면 추가검사시행(ex. MLPA등)을 권유드립니다.

Genomic analysis

- MLPA method: large deletion과 duplication을 정량화하여 보여줌
  - Duchenne muscular dystrophy의 *DMD* gene
  - spinal muscular dystrophy의 *SMN1* gene
  - CMT1A의 *PMP22* duplication/deletion
- Direct sequencing
  - 일반적인 missense, nonsense, splicing site, small del/in의 확인에 사용
  - 일반적으로 말하는 유전자검사
- 두 방식은 서로 보완적으로 원인 유전자의 흔한 돌연변이 유형에 따라서 시행순서가 다르다.
  - Ex) DMD: MLPA ⇒ Direct sequencing
    ABCD1: Direct sequencing ⇒ MLPA
- Next generation sequencing의 적용



Sanger's sequencing

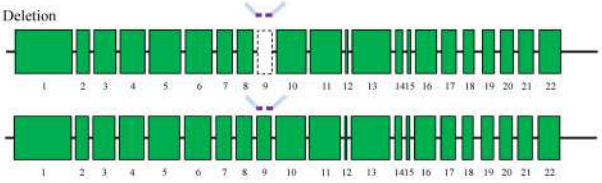


YONSEI UNIVERSITY

Sanger's sequencing

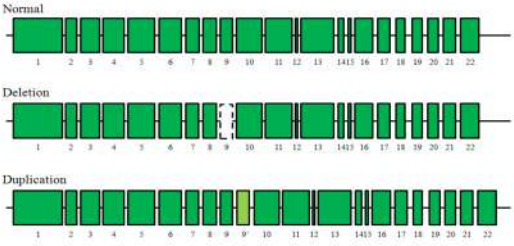
- Sequencing can be performed on RT-PCR derived cDNA from muscle RNA, or on genomic DNA.
- Complex rearrangements, or variants located deep into the large introns of the gene will not be detected.

Deletion



YONSEI UNIVERSITY

Copy number variation

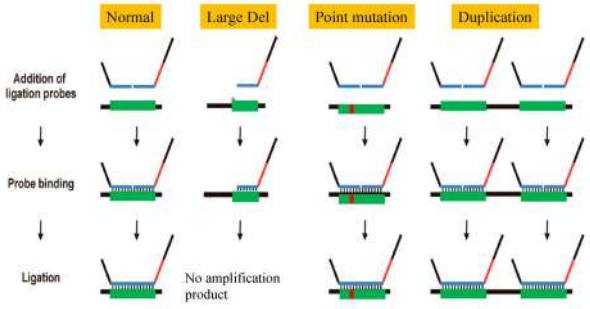


Available tests

- **MLPA(multiplex ligation-dependent probe amplification)**
- MAPH(multiplex amplification and probe hybridization)
- CGH array (comparative genomic hybridization array)

YONSEI UNIVERSITY

MLPA (multiplex ligation-dependent probe amplification)

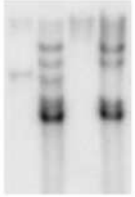


YONSEI UNIVERSITY

PCR + Southern blotting

- Trinucleotide repeats

1. Cut DNA using Restriction endonucleases
2. Electrophoresis on an agarose gel to separate them by size
3. Move the DNA from the gel onto the nitrocellulose (or, alternatively, nylon) membrane
4. Expose to a hybridization probe which is labelled so that it can be detected
5. After hybridization, the pattern of hybridization is visualized on X-ray film by autoradiography in the case of a radioactive or fluorescent probe



YONSEI UNIVERSITY

방법	보편적인 점돌연변이 (common point mutation)	드문 점돌연변이 (rare point mutation)	염색수변이 (copy number variation)	단순성 이염색체성 (segmental disomy)*	균형염색체 자리바꿈 또는 자리옮김 (balanced rearrange. or translocation)	반복확장 (repeat expansion)	분석민감도 (analytical sensitivity)*	분석특이도 (analytical specificity)*	검사시간	비용
Linkage analysis (commonly STRs)	0		0				Low	Low	Low	Low
FISH			0		0		Low	Low	Low	Low
Array CGH or virtual karyotyping			0	0			Average	Average	Average	Average
Genome-wide SNP microarray	0		0			0	Low	Low	Low	Low
Targeted PCR	0	0				0	High	High	Low	Low
Sanger's sequencing	0	0					High	High	Average-High	Average
Southern blot or MLPA			0			0	High	High	High	Low
Panel or pathway sequencing	0	0					Average	Low	Average	Average
WES or WGS	0	0	0†				Low	Low	High	High

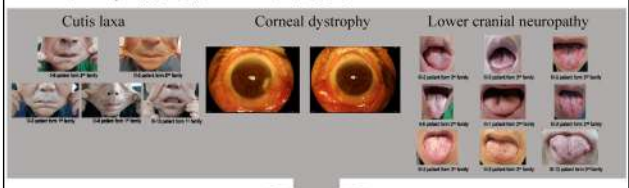
CGH: 비교유전체보합법(comparative genomic hybridization), FISH: 형광동소보합법(fluorescent in situ hybridization), MLPA: 다중결찰의존프로브증폭법(multiplex ligation-dependent probe amplification), SNP: 단일핵산염기다형현상(single-nucleotide polymorphism), STR: 단기일렬반복(short tandem repeat), WES: 전체엑솜염기서열분석(whole-exome sequencing), 전체유전자염기서열분석, WGS: 전체유전체염기서열분석(whole-genome sequencing)

YONSEI UNIVERSITY

Known point mutation

- Sanger sequencing

Ex) Hot-spot 있는 경우, Validation, 가족검사



c.640G>T variant in *GSN*

YONSEI UNIVERSITY

Copy number variation

- 형광동소보합법(fluorescence in situ hybridization; FISH)
- 단일염기다형성어레이 (SNP array)
- 비교유전체보합법(array comparative genomic hybridization; aCGH)
- 실시간중합효소연쇄반응(real-time polymerase chain reaction)
- 서던블롯검사(Southern blotting)
- 다발중합효소연쇄반응(multiplex polymerase chain reaction)
- 다중증폭프로브교배법(multiplex amplifiable probe hybridization, MAPH)
- **다중결찰의존프로브증폭법(multiplex ligation-dependent probe amplification, MLPA)**
- 차세대염기서열 분석법(next-generation sequencing; NGS)

YONSEI UNIVERSITY

Trinucleotide repeat disease

- **반복의 크기가 작은 경우**
  - 헌팅턴병(Huntington disease)
  - 중합효소연쇄반응(polymerase chain reaction, PCR)
    + 모세관 전기영동법(capillary electrophoresis)
- **반복의 크기가 큰 경우**
  - Myotonic dystrophy
  - 서던블롯(Southern blot)

YONSEI UNIVERSITY

Many targeted genes

- Next-generation sequencing
  (Massive parallel sequencing)

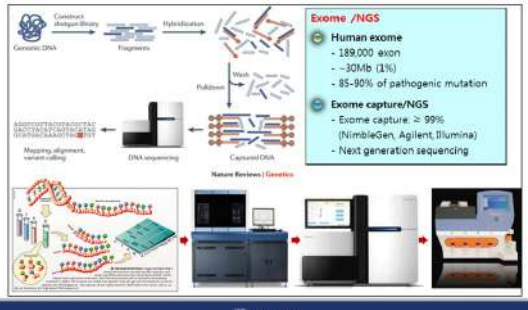
YONSEI UNIVERSITY

Sanger's sequencing vs NGS

- 클론(clone)을 얻는 과정을 단순화
- 대량 병렬(massively parallel)방식
- 염기서열분석방법이 다름

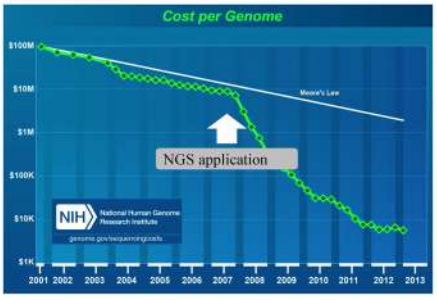
YONSEI UNIVERSITY

Next-generation sequencing



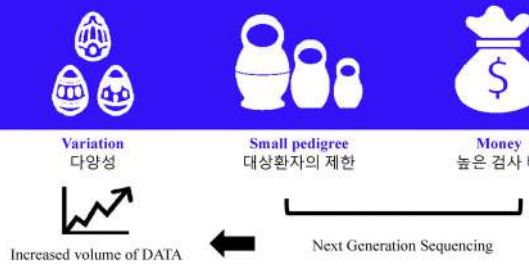
YONSEI UNIVERSITY

Application of Next-generation sequencing



YONSEI UNIVERSITY

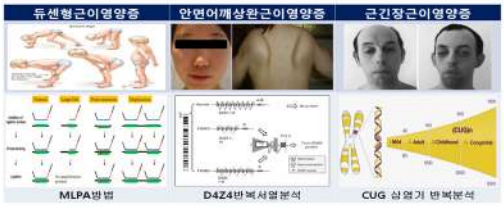
Limitation of conventional approach



YONSEI UNIVERSITY

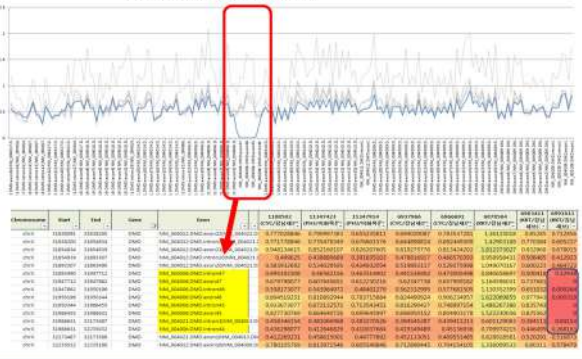
Next-generation sequencing - limitation

- 한 번에 읽을 수 있는 염기서열이 150 base pair로 제한적임
  - 반복염기가 많거나 GC 함량이 높아지면 error율이 증가
  - Trinucleotide repeat 또는 large duplication/deletion과 같이 정량적 분석이 필요한 원인유전자의 진단은 어려움.
- Intron 영역에 대한 해석을 위한 방법이 제한적임.



YONSEI UNIVERSITY

Deletion of exon 45-47



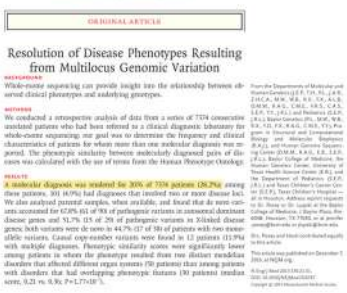
YONSEI UNIVERSITY

Whole-exome vs Whole-genome

	Whole-exome	Whole-genome
Depth	High	Low
Interpretation	Easy	Difficult
Handling	Easy	Difficult
Copy number variation	Possible	Relatively accurate

YONSEI UNIVERSITY

Molecular diagnosis of WES was rendered for 2076 of 7374 patients (28.2%).



ORIGINAL ARTICLE

Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation

N Engl J Med. 2017 376(1): 21–31

YONSEI UNIVERSITY

Diagnostic ratio of WGS: 41%

Official Journal of the American College of Medical Genetics and Genomics **ORIGINAL RESEARCH ARTICLE** | Genetics inMedicine

Open

Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test

Purpose: Genetic testing is an integral diagnostic component of pediatric medicine. Standard of care is often a time-consuming stepwise approach involving chromosomal microarray analysis and targeted gene sequencing panels, which can be costly and inconclusive. Whole-genome sequencing (WGS) provides a comprehensive testing platform that has the potential to streamline genetic assessments, but there are limited comparative data to guide its clinical use.

Methods: We prospectively recruited 103 patients from pediatric non-genetic subspecialty clinics, each with a clinical phenotype suggestive of an underlying genetic disorder, and compared the diagnostic yield and coverage of WGS with those of conventional genetic testing.

Results: WGS identified diagnostic variants in 41% of individuals, representing a significant increase over conventional testing results (24%, $P = 0.01$). Genes clinically sequenced in the cohort ($n = 1,226$) were well covered by WGS, with a median exonic coverage of 40× ± 8× (mean ± SD). All the molecular diagnoses made by conventional methods were captured by WGS. The 18 new diagnoses made with WGS included structural and non-exonic sequence variants not detectable with whole-exome sequencing, and confirmed recent disease associations with the genes *PIGG*, *RNF144A/FAC*, *TRIO*, and *UNC13A*.

Conclusion: WGS as a primary clinical test provided a higher diagnostic yield than conventional genetic testing in a clinically heterogeneous cohort.

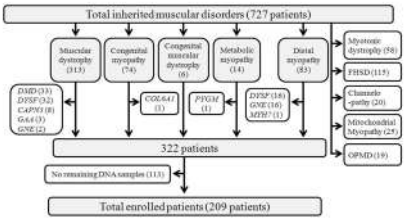
Genet Med advance online publication 3 August 2017

Key Words: copy number variation; next-generation sequencing; remodeling; diagnostics; whole-genome sequencing

Genet Med 2018; 20(4): 435–443

YONSEI UNIVERSITY

Our previous targeted sequencing



We identified 93 (36%) patients with pathogenic/likely pathogenic variants.

Park HJ et al. Clin Genet 2017;91:403-410

YONSEI UNIVERSITY

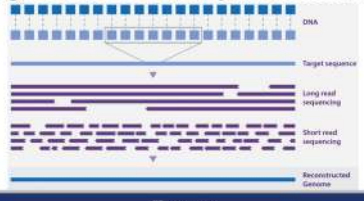
Experience in our institution

- Targeted sequencing for about 400 neuromuscular-associated genes
- **Diagnostic ratio**: 43.5% (128 of 294)

YONSEI UNIVERSITY

Long-read whole genome sequencing

- LRS allows for the retrieval of much longer (>10,000bp) sequencing reads than widely-used SRS systems (75-300bp).
- Read lengths of 10,000- 100,000bp are more common.



YONSEI UNIVERSITY

Variant and pathogenicity

© American College of Medical Genetics and Genomics **ACMG STANDARDS AND GUIDELINES** | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[15] and Heidi L. Rehm, PhD[16]; on behalf of the ACMG Laboratory Quality Assurance Committee

YONSEI UNIVERSITY

Database

YONSEI UNIVERSITY

Database

gnomAD

genome aggregation database

The Human Gene Mutation Database

at the Institute of Medical Genetics in Cardiff

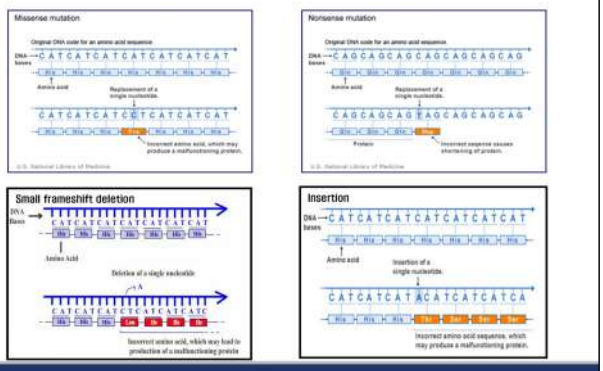
Leiden Muscular Dystrophy pages©

Center for Human and Clinical Genetics,

Leiden University Medical Center

YONSEI UNIVERSITY

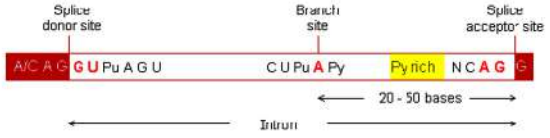
Type of variants



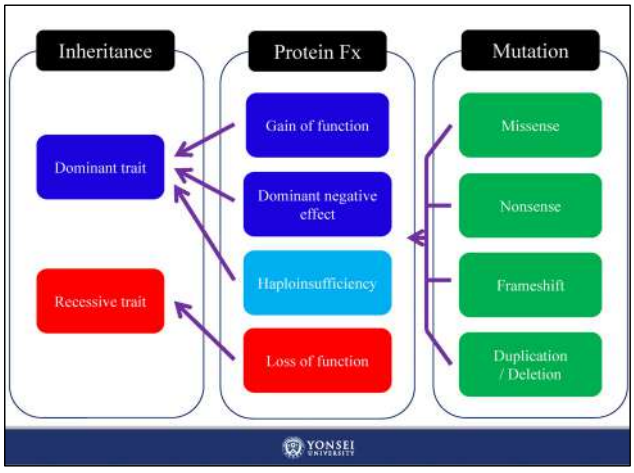
YONSEI UNIVERSITY

Type of variants

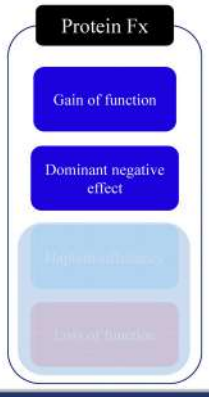
Splicing variants



YONSEI UNIVERSITY

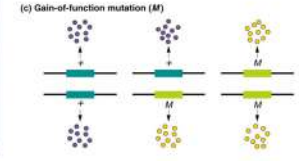


YONSEI UNIVERSITY

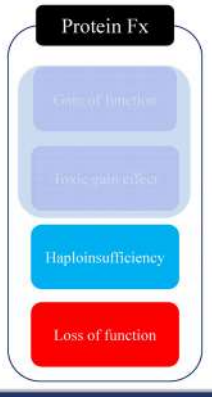


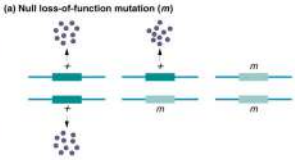
단백질의 양이 증가하면서 증상을 일으킴
Ex) *PMP22* duplication in CMT1A

손상된 단백질이 쌓이면서 증상을 일으킴
Ex) *Myotilin* in Myofibrillar myopathy



YONSEI UNIVERSITY





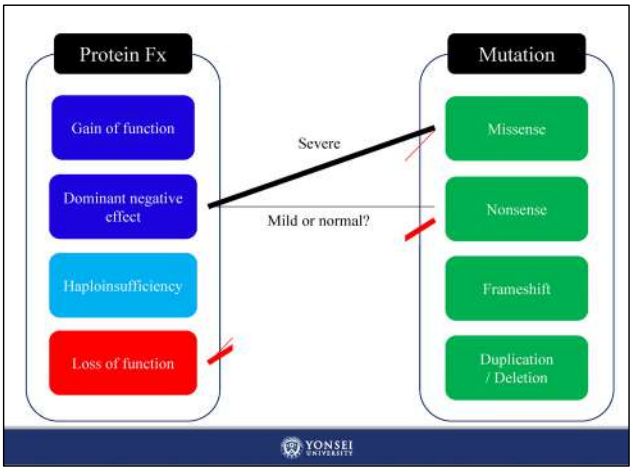
단백질 양이 감소하여서 증상이 나타남

Ex) *SMN1* in spinal muscular atrophy

일반적으로 50%이상의 단백손실이 있어야 증상이 나타남 그렇지 않은 경우를 Haploinsufficiency라고 일컬음.

Ex) channelopathy

YONSEI UNIVERSITY



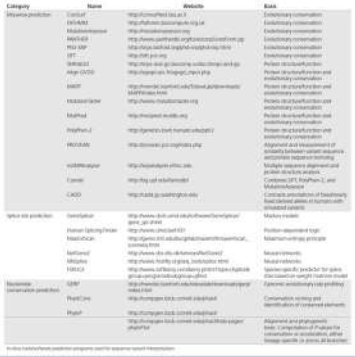
Heterogeneity in phenotype-genotype relationship

- Progressive external ophthalmoplegia를 일으키는 *POLG1* mutation은 autosomal dominant와 autosomal recessive로 모두 유전된다.
- COL6A1, COL6A2, COL6A3는 autosomal dominant일 경우 mild Bethlem myopathy로 autosomal recessive일 경우 severe Ullrich congenital muscular dystrophy로 …

=> Phenotype 및 Genotype이 다르다.

YONSEI UNIVERSITY

In-silico analysis



YONSEI UNIVERSITY

Summary

- Variant nomenclature
- Point variant vs Copy number variation vs Trinucleotide repeat
- Variant vs Pathogenicity
- Massively palliative sequenging

YONSEI UNIVERSITY