Molecular & Biochemical Basis of Common Genetic Disorders in Korea

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I. 인간 유전체 구조 (Human genome organization)

1. Nuclear Genome: 3,000 Mb, <30,000 genes

1) genes & gene related sequences: 25% of whole genome
   (1) coding DNA (exons): 1.1% of whole genome
   (2) noncoding DNA (introns): 24% of whole genome
   (3) OMIM (online mendelian inheritance in man)에 >10,000 유전자등록 (2004년), >1500 gene loci의 mutation이 인체 유전 질환과 연관, 이는 전체 유전자의 약 5%에 해당
2) extragenic DNA (intergenic): 75% of whole genome
   (1) unique or low copy DNA (~60%)
   3% of DNA; mRNA, rRNA, tRNA
   (2) highly repetitive
      ① 5~10% of DNA
      ② Alu repeat는 인체 DNA 평균 4 kb 마다 존재
      ③ SINES (short interspersed element)
      LINES (long interspersed element)
      ④ microsatellites, minisatellites; di, tri, tetra, penta-nucleotide
   (3) moderately repetitive (15~20%)
      ① 수십-수천 copy
      ② rRNA, tRNA, histone, antibody 등의 유전자
      ③ telomere염기서열 (TTAGGG); 2,000 copies

2. Mitochondrial genome: 16.6 kb, 37 genes

1) 2 rRNA genes
2) 22 tRNA genes
3) 13 polypeptide encoding genes

II. 인간 유전자 구조 (Human gene structure)

1. mRNA processing
2. intron-exon boundary
3. How do we know it is a gene?
4. What is the average size of gene?
5. How densely are genes located on each chromosome?

III. 인체유전자의 돌연변이 (Nature of mutations)

1. 돌연변이의 종류

1) germinal mutation vs somatic mutation
2) spontaneous mutation vs induced mutation
3) coding region vs non-coding region
4) substitution과 frame shift
   (1) 73%의 base substitution은 missense mutation
       23%의 random base substitution은 silent mutation
   (2) gene rearrangement (삽입, 결실--unequal crossing over)
   (3) 질환의 예:
5) unstable trinucleotide repeats
   (1) Fragile-X 증후군
   (2) Myotonic dystrophy
   (3) Huntington's disease
   (4) Spinocerebellar ataxia
   (5) 기타 질환들

2. 돌연변이의 원인

1) chemical mutagenesis
2) radiation mutagenesis
3) thwarting mutations
   (1) 손상된 DNA의 복구 (DNA repair system)
   (2) 비정상 DNA repair syndrome
       ① xeroderma pigmentosum
       ② ataxia telangiectasia
       ③ Bloom syndrome
       ④ Werner syndrome
       ⑤ Fanconi anemia

3. Concept of genomic disorders and genetic disorders

1) simple mutations
2) genetic mechanisms which result in sequence exchanges between repeats
3) pathogenic potential of repeated sequences

IV. 유전자돌연변이와 단백기능의 변화, 표현형 (임상상)의 변화 (genotype-phenotype relationship)

기 능	돌연변이에 의해 초래되는 기능이상 단백 (질병)	유전방식
효소		
아미노산	· phenylalanine hydroxylase (페닐케톤요증)	상염색체열성
탄수화물	· galactose-1-phosphate uridyl transferase (갈락토스혈증)	상염색체열성
유기산	· methylmalonyl-CoA mutase (메틸말로닐산혈증)	상염색체열성
지질	· medium chain acyl CoA dehydrogenase (MCAD 결핍증)	상염색체열성
복합지질	· hexosaminidase A (Tay-Sachs병)	상염색체열성
퓨린	· adenosine deaminase (중증복합면역결핍증)	상염색체열성
포르피린	· porphobilinogen deaminase (급성간헐성 포르피리아)	상염색체우성
이동과 축적		
Interorgan	· hemoglobin (thalassemia, hemoglobin variant)	상염색체열성
Organell막	· lysosomal cystine transport protein (cystinosis)	상염색체열성
세포내이동	· copper transport protein (Menkes 병)	성염색체열성
상피세포막	· protein involved in chloride transport (cystic fibrosis)	상염색체열성
세포, 기관의 구조		
세포외	· type I, II collagen (골형성부전)	상염색체열성, 우성
	· type III collagen (Ehlers-Danlos 증후군 IV형)	상염색체우성
세포막, cytoskeleton	· red cell membrane skeleton protein, spectrin (구상적혈구증)	상염색체우성
	· dystrophin (Duchenne/Becker 형 근이양증)	성염색체열성
Organelle	· protein required for peroxisome biogenesis (Zellweger 증후군)	상염색체열성
세포외 homeostasis		
면역체계	· proteins of the complement system (C3 결핍)	상염색체열성, 우성

기 능	돌연변이에 의해 초래되는 기능이상 단백 (질병)	유전방식
지혈	· factor VIII (혈우병A)	성염색체열성
Protease억제	· alpha1 antitrypsin (간질환, 폐기종)	상염색체열성
발달관련 유전자발현	(Developmental gene expression)	
전사인자	· PAX6, a homeodomain transcription facto (aniridia)	상염색체우성
	· WT1, zinc finger transcriptional factor (Wilms' tumor)	상염색체우성
Signaling molecules	· sonic hedgehog (holoprosencephaly)	상염색체우성
Signaling receptors	· FGFR3 receptors (achondroplasia)	상염색체우성
Ribosomal proteins	· S19 ribosomal protein (Diamond-Blackfan anemia)	상염색체우성
성장과 분화의 조절		
종양억제	· Rb protein (retinoblastoma, osteosarcoma)	상염색체열성
암유전자	· c-abl proto-oncogene (만성골수성 백혈병)	체세포돌연변이
	· the Ret receptor tyrosine kinase (MEN2)	상염색체우성
세포간 대사 및 communication		
Cell-cell channels	· connexin 43 gap junction protein (심장기형)	상염색체열성
	· connexin 26 gap junction protein (nonsyndromic deafness)	상염색체열성
광수용체	· rhodopsin (one form of AD retinitis pigmentosa)	상염색체우성
	· green, red light opsins (X-linked color blindness)	성염색체열성
호르몬	· growth hormone (왜소증)	상염색체열성
	· insulin (rare form of adult onset diabetes mellitus)	상염색체우성
호르몬 수용체	· vitamin D receptor, DNA binding protein (vitamin D dependent rickets type II)	상염색체열성
	· androgen receptor (testicular feminization)	성염색체열성
	· insulin receptor (leprechaunism)	상염색체열성
	· vasopressin V2 receptor (diabetes insipidus)	성염색체열성
Signal transducer	· stimulatory guanine nycleotide-binding protein of adenylate cyclase (pseudohypoparathyroidism)	상염색체우성
	· defective cyclic AMP response to vasopressin (diabetes insipidus)	성염색체열성
Metabolite 수용체	· low density lipoprotein receptor (familial hypercholesterolemia)	상염색체우성

V. 단일 유전자 질환의 유전자 분석

1. 시료의 종류:

blood samples, mouth washes or buccal scrapes, chorionic villi biopsy samples, one or two cells removed from 8-cell stage embryos, archived pathological specimens, Guthrie cards, hair follicles 등

2. 알려진 특정 돌연변이의 검사방법 (genotyping):

PCR-RFLP, dot blot 또는 gene chip을 이용한 allele specific oligonucleotide hybridization (ASO) on a dot blot or gene chip, oligonucleotide ligation assay (OLA), allele specific PCR amplification (ARMS test), fragment analysis, dHPLC, Mass array, pyrosequencing, invader assay

3. 알려지지 않은 돌연변이 발견을 위한 scanning 방법 (identification of novel mutation)

1) Southern blot: gene rearrangement(large insertion, deletion)
2) sequencing: 비용이 들고 해석이 어려운 점이 있으나 유전자 완전 분석가능
3) heteroduplex gel mobility: 단순, 저렴하나, 크기가 <200 bp인 경우, small insertion과 deletion, 감수성이 낮음
4) denaturing HPLC: 빠르고 high throughput, 대량처리 가능, expensive equipment
5) SSCP: 단순, 저렴, limited sensitivity, 크기가 <200 bp인 경우
6) DGGE (denaturing gradient gel electrophoresis): 예민도가 크나 primer design이 중요하고, expensive primers
7) mismatch cleavage (chemical, enzymatic): 예민도는 크나 과정이 어렵고 복잡하며 toxic chemicals 사용
8) protein truncation test: nonsense, frame shift mutation 검색에만 용이하며 RNA 사용, 과정이 복잡
9) oligonucleotide arrays (gene chip)빠르고 대량분석 가능, 고비용
10) high throughput DNA sequencing: 대량분석 가능하나 비싸고 해석의 어려움

VI. Molecular and biochemical bases of common genetic disorders in Korea:

Mutation and functional study data on following disorders will be presented at the symposium.

1. Inherited metabolic disorders; urea cycle defects, Wilson disease, lysosomal storage disease, congenital adrenal hyperplasia
2. Skeletal dysplasia: achondroplasia, craniosynostosis syndrome
3. Neuromuscular disorders: DMD/BMD, spinal muscular atrophy
4. Triplet repeat expansion disorders: spinocerebellar ataxia, fragile-X syndrome, myotonic dystrophy, Kennedy disease, Huntington's disease, DRPLA
5. Neurogenetic disorders; metachromatic leukodystrophy
6. Mitochondrial disorders: MELAS, MERRF, LHON, Kearn-Sayers syndrome

7. DNA testing for contiguous gene syndromes의 예
1) CATCH22 (DiGeorge/velocardiofacial) syndromes
2) Prader-Willi/Angelman syndrome
3) Williams syndrome

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