GENOMIC ANALYSIS ON DUCHENNE MUSCULAR DYSTROPHY

Satarupa Banerjee
B.Sc Biotechnology
Indian Biological Sciences and Research Institute (IBRI), Noida, Delhi, India

Abstract - Aim And Objective :
To study the details about the structural and functional Genomic Analysis of Human Duchenne Muscular Destrophy disease and compare it with 3 other organisms like Pig, Mouse and Chimpanzee to find out one of the cause of drop down of the population of Chimpanzee. To compare a single sequence of Duchene Muscular Destrophy Gene to its entire database and to compare a partial sequence to the whole and to compare a short sequence to a large one and to compare a query sequence against a database, FASTA and BLAST, and Primer BLAST are done and then Phylogenetic tree and Gene Graphics are done from NCBI. Take out the Melting Point of forward and reverse Primer Pair 1 by Super Computing Facility For Bioinformatics and Computational Biology. DNA Complement and DNA Reverse Complement and ORF search has been done by Sequence Analyzer Bioeezee-Marine Bioinformatics. To generating, formatting and analysis the DNA sequencing of responsible gene of DMD, the Sequence Manipulation Suite is done. Find the Linear sequence by NEB enzyme (non overlapping ORF), the NEB Cutter has been used.

Introduction :-

Gene Cards Summary for DMD Gene:

DMD (dystrophin) is a protein-coding gene. Diseases associated with DMD include *duchenne muscular dystrophy*, and *becker muscular dystrophy*.

GenomicLocation:

Entrez Gene cytogenetic band: **Xp21.2** Ensembl cytogenetic band: **Xp21.1** *HGNC* cytogenetic band*:* **Xp21.2**

The gene of dystrophin is one of the biggest gene to be seen in nature, of about 2.4 Mb. The gene was identified through a positional cloning approach, targeted at the isolation of the gene responsible for Duchenne Muscular Dystrophy (DMD) and it is a recessive, fatal, X-linked form of muscular dystrophy/ disorder occurring at a frequency of about 1 in 3,500 new-born males which results in muscle degeneration and eventual death. In general, DMD patients carry mutations which cause premature translation termination (nonsense or frame shift mutations) in the dystrophin gene at locus Xp21, the largest gene located on the human X chromosome (Xp21.2-p21.1), which codes for the protein dystrophin, an important structural component within muscle tissue that provides structural stability to the dystrogly can complex (DGC) of the cell membrane containing at least eight independent, tissue-specific promoters and two polyA-addition sites.Furthermore, dystrophin RNA is differentially spliced, producing a range of different transcripts, encoding a large set of protein isoforms.

While both genders can convey the change or mutation, females once in a while display indications of the infection. DMD is acquired in an X-connected pattern in recessive form which dangers incorporate a family ancestry of Duchenne muscular dystrophy. Females will be transporters for the infection while males will be influenced ie a female bearer will be unconscious that they convey a change until they have an influenced son. Males have just 1 X chromosome, so 1 duplicate of the transformed gene will cause DMD. Fathers can't pass X-connected characteristics on to their children thus an unaffected dad will either pass an ordinary Y to his child or a typical X to his little girl and the change is transmitted by the mother who is a transporter, and along these lines 1 of her 2 X chromosomes has a DMD transformation. The child of a transporter mother has a half possibility of acquiring the flawed gene from his mom. The little girl of a transporter mother has a half shot of being a bearer and a half possibility of having two ordinary duplicates of the gene.

Material And Methodology :Material:

Tools, Softwares and Data Bases:

- National Center for Biotechnology Information NCBI: The National Center for Biotechnology Information (NCBI)
- Nucleotide Blast NCBI
- Fasta Format NCBI
- Pick Primar NCBI
- The Sequence Manipulation Suite (bioinformatics sms): NATIONAL INSITIUTE OF OCEAN TECHNOLOGY
  - DNAComplement.
  - ORF Searching:
- NEBcutter V2.0. Super Computing Facility for Bio-informatics & Computational Biology.

Methodology:

Comparison study of Human with other 3 organisms like pig, mouse, and chimpanzee.

1. Study the human DMD disease and its details like responsible gene its symptoms, diagnosis and treatment.
2. Find Fasta format, Blast, Phylogenetic Tree, Gene Bank, Primar Blast,Gene Graphics of responsible gene of human DMD from NCBI .
3. Find the melting point of primar pair 1(forward and reverse) by SCF Bio.
4. Find the DNA Complement and reverse DNA Complement and ORF search from Sequence analyzer Bioeeze from Marine Bioinformatics.
5. Find the Codon Plot, Codon Usages, CpG Island, DNA Pattern Find, DNA Stats, ORF Finder, Positional Base Frequency, Restriction Summary, Simple Plot, Test Code, and Translate from DNA Analysis from The Sequence Manupulation Suite (Bioinformatics SMS).
6. Take out the large and non overlapping ORF from NEB Cutter V2.0.

Result And Discussion:

Result

NCBI Result:

SPECIES	GENE BANK/ACCESSION NO	QUERY COVER	MAX SCORE	TOTAL SCORE	IDENT	E-VALUE	MAX" SEQUENCE DIFFERENCE
Homo sepience	AF209189.1	100%	4403	4403	100%	0.0	.75
Chimpanzee	CR962120.1	100%	3816	3816	96%	0.0	.75
Pig	CU914583.5	20%	483	483	84%	8e-132	.75
Mouse	BX000479.11	21%	252	252	76%	2e-62	.75

SCF Bio Result:

species	Melting Point-Forward Primer Pair	Melting Point-Reverse Primer Pair	DNA Concentration	Sequence Length
Homo sepience	69.88c	68.77c	.0001M	70
Chimpanzee	69.51c	69.14c	.0001M	50
Pig	68.41c	65.15c	.0001M	50
Mouse	70.24c	67.30c	.0001M	50

NEB Cutter V2.

species	GC Content	AT Content
Homo sepience	36%	64%
Chimpanzee	36%	64%
Pig	35%	65%
Mouse	35%	65%

The Sequence Manipulation Suite:

Species	CpG island	DNA Pattern Find	Positional Base Frequency	Test Code Value	Simple Plot
Homo sepience	No Found	Negative Value-Reverse Strand	Divergence Value 112.67	.369- noncoding	
Chimpanzee	No Found	Negative Value-Reverse Strand	Divergence Value 74	.349-noncoding	
Pig	Found	Negative Value-Reverse Strand	Divergence Value 824	.314- noncoding	
Mouse	Found	Negative Value-Reverse Strand	Divergence Value 504	.337-noncoding	

MarineBioInformatics:

species	ORF Search(starting from)	DNA Complement
Homo sepience	“ctgcagtgtt”	“CTGCAGTGTT”
Chimpanzee	“tttcagggac”	“TTTCAGGGAC”
Pig	“gatctctttc”	“GATCTCTTTC”
Mouse	“cattatgagt”	“CATTATGAGT”

Discussion

Results for 2384 residue sequence "gi|8118038|gb|AF209189.1| **Homo sapiens** dystrophin(DMD) gene, intron 44 P20b sequence" starting "ctgcagtgtt". Linear Sequence: gi8118038gbAF209.

The dystrophin gene is the biggest gene seen in nature, estimating 2.4 Mb. The gene was recognized through a positional cloning method, directed at the disengagement of the gene in charge of Duchenne Muscular Dystrophy (DMD) and it is a passive, deadly, X-connected type of muscular dystrophy/issue happening at a recurrence of around 1 out of 3,500 new-conceived guys which results in muscle degeneration and possible demise. In general, DMD patients convey transformations which cause untimely interpretation end (rubbish or edge move changes) in the dystrophin gene at locus Xp21, the biggest gene situated on the human X chromosome (Xp21.2-p21.1), which codes for the protein dystrophin, a significant basic part inside muscle tissue that gives basic security to the dystroglycan complex (DGC) of the cell layer containing in any event eight free, tissue-explicit advertisers and two polyA-expansion sites. Furthermore, dystrophin RNA is differentially joined, delivering a scope of various transcripts, encoding a huge arrangement of protein isoforms.

While both genders can convey the change or mutation, females seldom display indications of the infection. DMD is acquired in a X-connected passive example which dangers incorporate a family ancestry of Duchenne muscular dystrophy. Females will be transporters for the sickness while guys will be influenced ie a female bearer will be unconscious that they convey a transformation until they have an influenced son.Males have just 1 X chromosome, so 1 duplicate of the changed gene will cause DMD. Fathers can't pass X-connected qualities on to their children thus an unaffected dad will either pass an ordinary Y to his child or a typical X to his little girl and the transformation is transmitted by the mother who is a bearer, and along these lines 1 of her 2 X chromosomes has a DMD change. The child of a bearer mother has a half possibility of acquiring the deficient gene from his mom. The little girl of a transporter mother has a half shot of being a bearer and a half possibility of having two typical duplicates of the gene.

Location: Xp21.2 Exon count: 87

Annotation release	Status	Assembly	Chr	Location
107	current	GRCh38.p2 (GCF_000001405.28)	X	NC_000023.11 (31119219..33339609, complement)
105	previous assembly	GRCh37.p13 (GCF_000001405.25)	X	NC_000023.10 (31137345..33357726, complement)

ChromosomeXNC_000023.11

Compare with Others 3 Organisms

1. **Pan troglodytes(Chimpanzee) chromosome(X BAC PTB065B04)**

Results for 2608 residue sequence "gi|66992828:7866-10473 **Pan troglodytes** chromosome X BAC PTB-065B04, complete sequence" starting "tttcagggac". And Linear Sequence: gi669928287866.

2. **Mouse DNA sequence from clone RP23139I16 on chromosome X**

Results for 145084 residue sequence "gi|29125339|emb|BX000479.11| **Mouse** DNA sequence from clone RP23-139I16 on chromosome X, complete sequence" starting "cattatgagt". Linear Sequence: gi29125339embBX0.

Pig CH242-239D18 on chromosome X

Results for 154317 residue sequence "gi|226373104|emb|CU914583.5| **Pig** DNA sequence from clone CH242239D18 on chromosome X, complete sequence" starting "gatctctttc". Linear Sequence: gi226373104embCU.

Marine BioInfo

Homo sapiens dystrophin (DMD) gene, intron 44 P20b sequence which Accession No is AF209189.1, Results for 2384 residue DNA sequence"gi|8118038|gb|AF209189.1| starting from "CTGCAGTGTT" and ORF search is “ctgcagtgtt” from Marine Bioinformatics.

SCF Bio

Melting point of Homosepience is 68.77c (Reverse), 69.88c (Forward) where as Chimpanzy is 69.14c (Reverse), 69.51c (Forward), Mouse is 67.30c (Reverse), 70.24(Forward), and Pig is 65.15c (Reverse) , 68.41(Forward).

Everybody has same Sequence length of 50 and DNA concentration is .0001M.

NEB Cutter

Everybody has same PH value i.e. 7.9. GC and AT content are same of human and chimanzee i.e.AT-64% and GC-36% and again Pig and Mouse have same AT, GC content i.e AT-65% and GC-35%.

Bioinformatics SMS

In homo sapience and Chimpanzee , no CpG Island were found but in Mouse and Pig, CpG Island were found. In case of DNA Pattern Find, the value of all of them is negative and Strand is reverse and position is 5'base in the pattern. The divergence value (The positional base frequency) of Human is 112.67 where as Chimpanzee has 74, Mouse has 504, and Pig has 824. The Test code value of Human is 0.369 where as Chimpanzee has 0.349, Pig has 0.314 and Mouse has 0.337 which are all non coding.

NCBI

Query cover of Human is 100% which is match with Chimpanzee but in Pig it is 20% and in Mouse it's 21%. The E-Value of Human and Chimpanzee is 0.0 and Pig and Mouse have 8e-132 and 2e-62 respectively. The maximum score and total score of Human is 4403 and Chimpanzee has 3816, Mouse has 252 and Pig has 483. The Ident of Human is 100% where as Chimpanzee is 96%, Pig is 84% and Mouse is 76%.

Conclusions:-

According to the above conversation, it's reasoned that the Human Duchenne Muscular Destrophy infection is additionally hazardous for Chimpanzees. Chimpanzees, generally called chimps, have a place with the Hominidae family. They are known to have advanced in a similar area, equivalent to us humans. Inside the tropical timberlands, they for the most part live in open savanna forests, meadows and rainforests. As indicated by certain appraisals, chimpanzee populaces have dropped to one tenth of what they were toward the beginning of the twentieth century. Reasons that present real dangers to this imperiled species are enormous annihilation of their common natural surroundings and this sickness as well. It ought to be controlled to spare them and counteract to be endangered.

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