



CURSO DE FORMACIÓN CONTINUADA

de las Profesiones Sanitarias de la Comunidad de Madrid - 1,9 Créditos

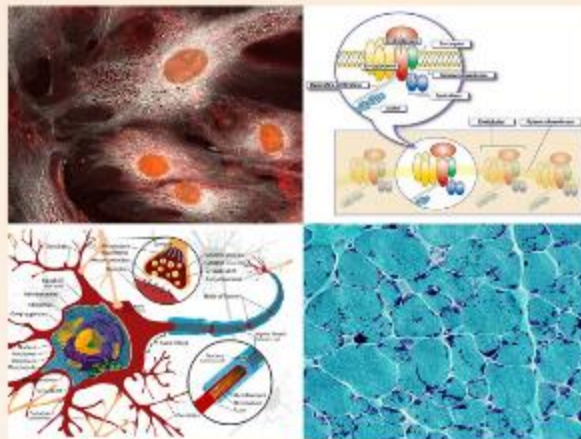


Hospital Universitario
Ramón y Cajal

Comunidad de Madrid



Enfermedades Musculares en la Infancia y Adolescencia (XII)



26 y 27 de Marzo de 2015

Salón de Actos, Planta 0 centro.
Hospital Universitario Ramón y Cajal

Inscripción: GRATUITA, enviando un correo
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Dr. A. Pedrera Mazarro

Neurología infantil S. de Pediatría.

Neurología infantil S. de Pediatría.

Servicio de Neurofisiología clínica



Uso de técnicas de NGS (Next Generation Sequencing) en el diagnóstico de las miopatías

Dr. A. Jiménez Escrig S. de Neurología Hospital Ramón y Cajal

Conflictos de interés



Jamás aceptaría pertenecer a un club que me admitiera como socio.
Groucho Marx.

Inbox: [CFP] Next Generation Sequencing (5 of 16)

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Date: Wed, 25 Mar 2015 20:23:55 +0100 [03/25/2015 08:23:55 PM CET]

From: bakic@intechopen.com

To: adriano.jimenez@hrc.es

Subject: [CFP] Next Generation Sequencing

Headers: [Show All Headers](#)

Dear Dr. Jimenez-Escrig,

I hope to find you well.

I would like to inform you that, based on your publishing history, you are invited to contribute a chapter to a new book project under the working title "Next Generation Sequencing". This book will be published by InTech Open Access publisher, under the editorship of Dr. J.K. Kulski.

The book will be an Open Access publication made available through InTech's reading platform and indexed in scientific databases.

To learn more about the project (suggested topics, deadlines and the publishing process) and to register your participation, please visit:

<http://www.intechopen.com/welcome/674916bc6a4ef97379f637d6f194e8c3/adriano.jimenez@hrc.es>

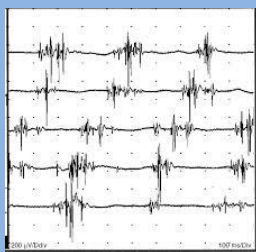
Please notice that an Article Processing Charge of 670 euros will be applied to cover the Open Access publishing process expenses.

We hope that you will be able to join this project and I will be happy to help with any questions you might have.

Cordially,

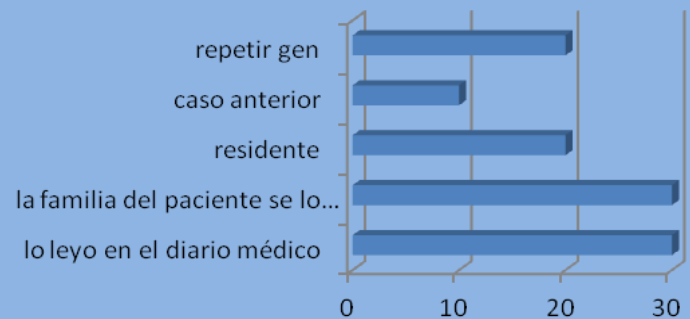
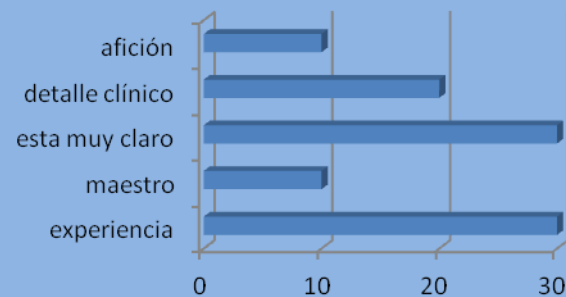
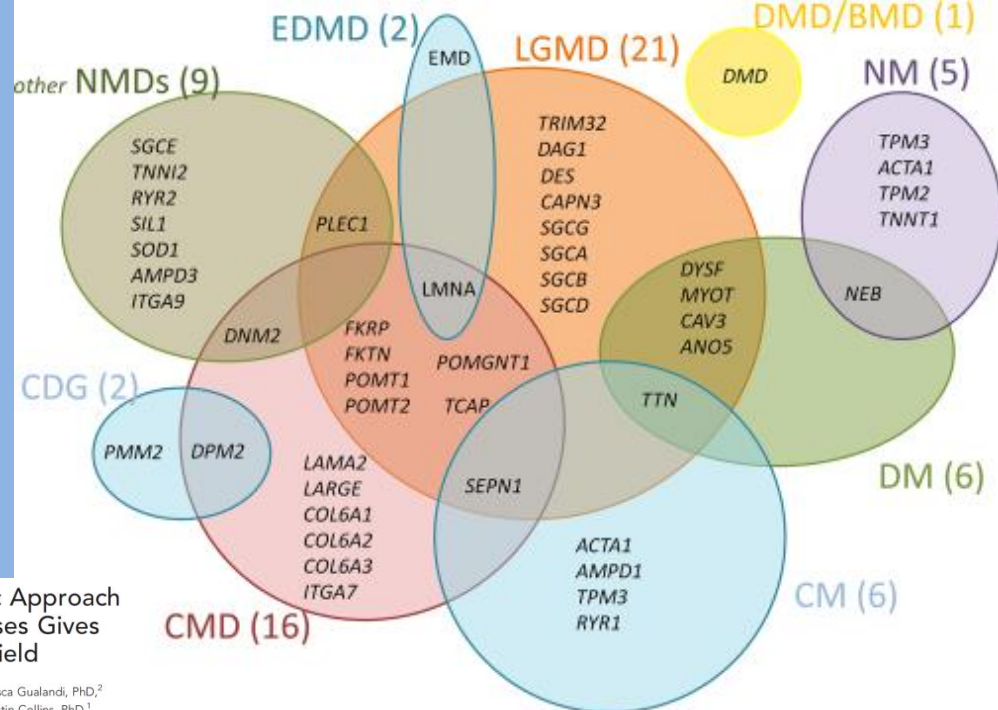
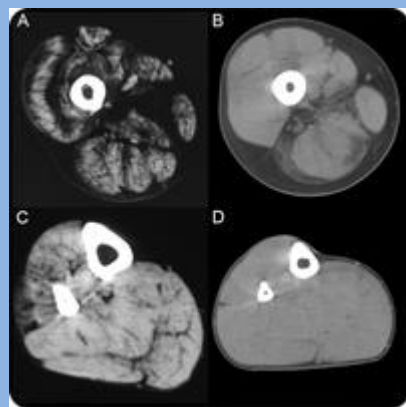
Sandra Bakic
Publishing Process Manager

E: bakic@intechopen.com

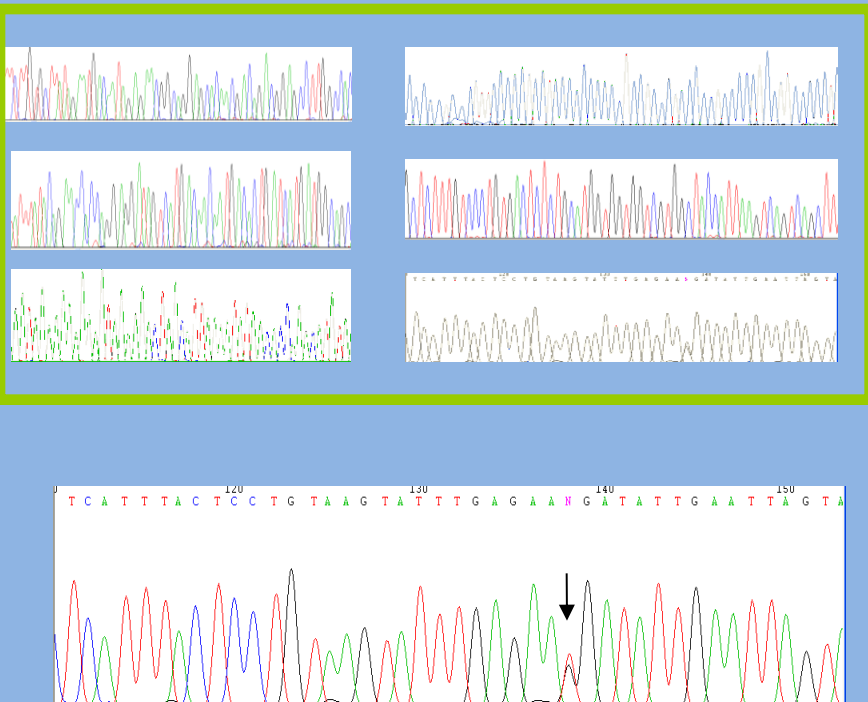
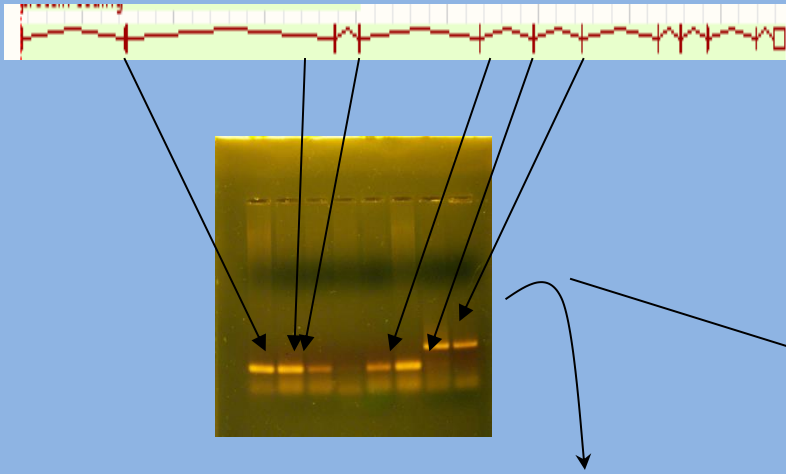


A Comprehensive Genomic Approach for Neuromuscular Diseases Gives a High Diagnostic Yield

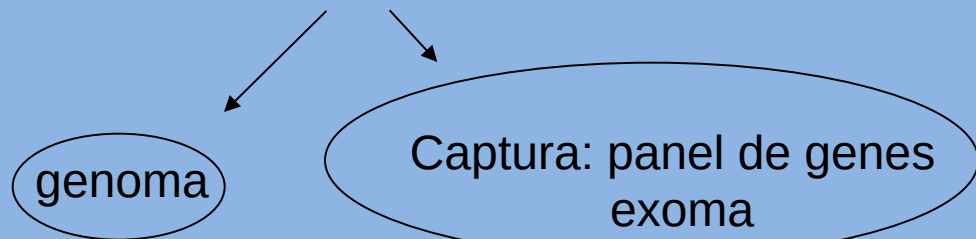
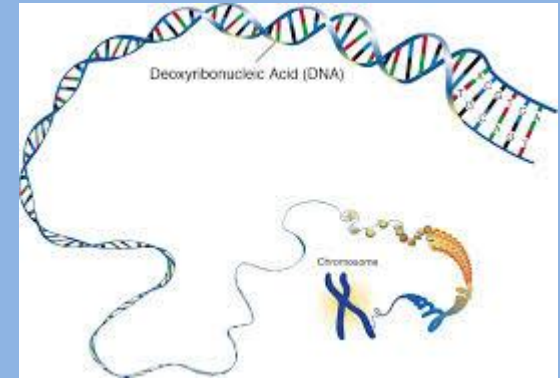
Arunkanth Ankala, PhD,¹ Cristina da Silva, MS,¹ Francesca Gualandi, PhD,²
Alessandra Ferlini, PhD,² Lora J. H. Bean, PhD,¹ Christin Collins, PhD,¹
Alice K. Tanner, PhD,¹ and Madhuri R. Hegde, PhD¹



SECUENCIACION POR SANGER



Next Generation Sequencing (NGS)



SECUENCIACION SANGER



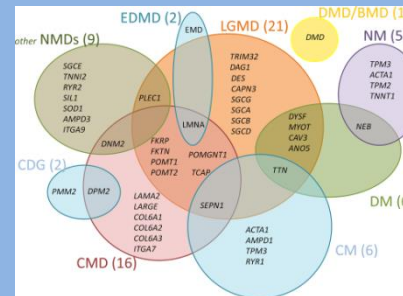
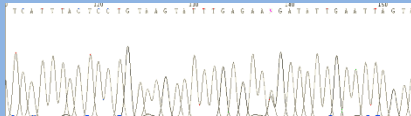
500\$

SECUENCIACION DE NUEVA GENERACION

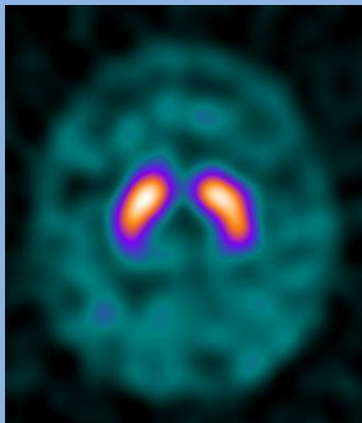


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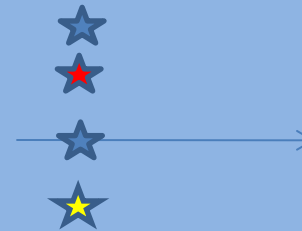
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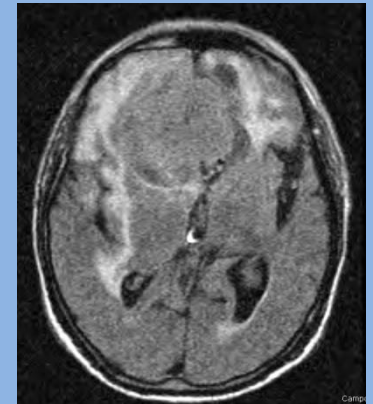
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4 dias



4 dias



OBJETIVOS

1) DESCRIPCION DE LA NGS

2) PRESENTACION DE CASOS

3) DISCUSION



bioinformática

16G RAM/4 TB

```
@IRIS:7:1:17:394#0/1
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+
aaabaa`]baaaaa_aab]D^^`b`aYDW]abaa`^
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+
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+
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+
abaaaaa`a````^aaaaa`_]aaa`aaa__a_X]``
.....
.....
```

fastq, fq
Exoma 5-10

Genoma 500 G



PIPELINE

1) ALINEACION CON GENOMA DE REFERENCIA → SAM, BAM (BWA, BOWTIE, NOVOALING, SOAP,...)

```
1:497:R:-272+13M17D24M 113 1 497 37 37M 15 100338662 0 CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG 0;====9;>>>>=>>>>>>
19:20389:F:275+18M2D19M 147 1 17919 0 18M2D19M = 17644 -314 GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT ;44999;499<8<8<<<8
9:21597+10M2I25M:R:-209 83 1 21678 0 8M2I27M = 21469 -244 CACCACATCACATATACCAAGCCTGGCTGTGTCTTCT <;9<<5><<<<<<<<<<<<
. . .
```

2) VARIANT CALL → VCF (GATK, FREEBAYES,...)

```
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20 2300608 rs84825 C T . PASS . GT:GP 0/1:0.03,0.97,0

20 2301308 rs84823 T G . PASS . GT:PL 1/1:10,5,0
....
```

3) ANOTACION (ANNOVAR, KGGSEQ)

1	Chr	Start	End	Ref	Alt	Func.refGene	Gene.refGene	GeneDetail.refGene	ExonicFunc.refGene	AAChange.refGene	cytoBand	genomicSu
2	1	948921	948921	T	C	UTR5	ISG15	NM_005101:c.-33T>C	.	.	1p36.33	.
3	1	1404001	1404001	G	T	UTR3	ATAD3C	NM_001039211:c.*91G>T	.	.	1p36.33	Score=0.90
4	1	5935162	5935162	A	T	splicing	NPHP4	NM_015102:exon22:c.2818-2T>A	.	.	1p36.31	.
5	1	162736463	162736463	C	T	intronic	DDR2	.	.	.	1q23.3	.
6	1	84875173	84875173	C	T	intronic	DNASE2B	.	.	.	1p31.1	.
7	1	13211293	13211294	TC	-	intergenic	HNRNPCP5,PRAMEF3	dist=26967;dist=116902	.	.	1p36.21	Score=0.95
8	1	11403596	11403596	-	AT	intergenic	UBIAD1,PTCHD2	dist=55105;dist=135699	.	.	1p36.22	.
9	1	105492231	105492231	A	ATAAA	intergenic	LOC100129138,NONE	dist=872538;dist=NONE	.	.	1p21.1	.
10	1	67705958	67705958	G	A	exonic	IL23R	.	nonsynonymous SNV	IL23R:NM_1447	1p31.3	.
11	2	234183368	234183368	A	G	exonic	ATG16L1	.	nonsynonymous SNV	ATG16L1:NM_19	2q37.1	.
12	16	50745926	50745926	C	T	exonic	NOD2	.	nonsynonymous SNV	NOD2:NM_0221	16q12.1	.

[illegible]



bioinformática

16G RAM/4 TB

```
@IRIS:7:1:17:394#0/1
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+
aaabaa`]baaaaa_aab]D^^`b`aYDW]abaa`^
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+
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+
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CATATTGTAGGTTGGATCTCGAAAGATATGAAAGAT
+
abaaaaa`a````^aaaaa`_]aaa`aaa__a_X]``
.....
.....
```

fastq, fq
Exoma 5-10

Genoma 500 G



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19:20389:F:275+18M2D19M 147 1 17919 0 18M2D19M = 17644 -314 GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT ;44999;499<8<8<<<8
9:21597+10M2I25M:R:-209 83 1 21678 0 8M2I27M = 21469 -244 CACCACATCACATATACCAAGCCTGGCTGTGTCTTCT <;9<<5><<<<<<<<<<<<
...

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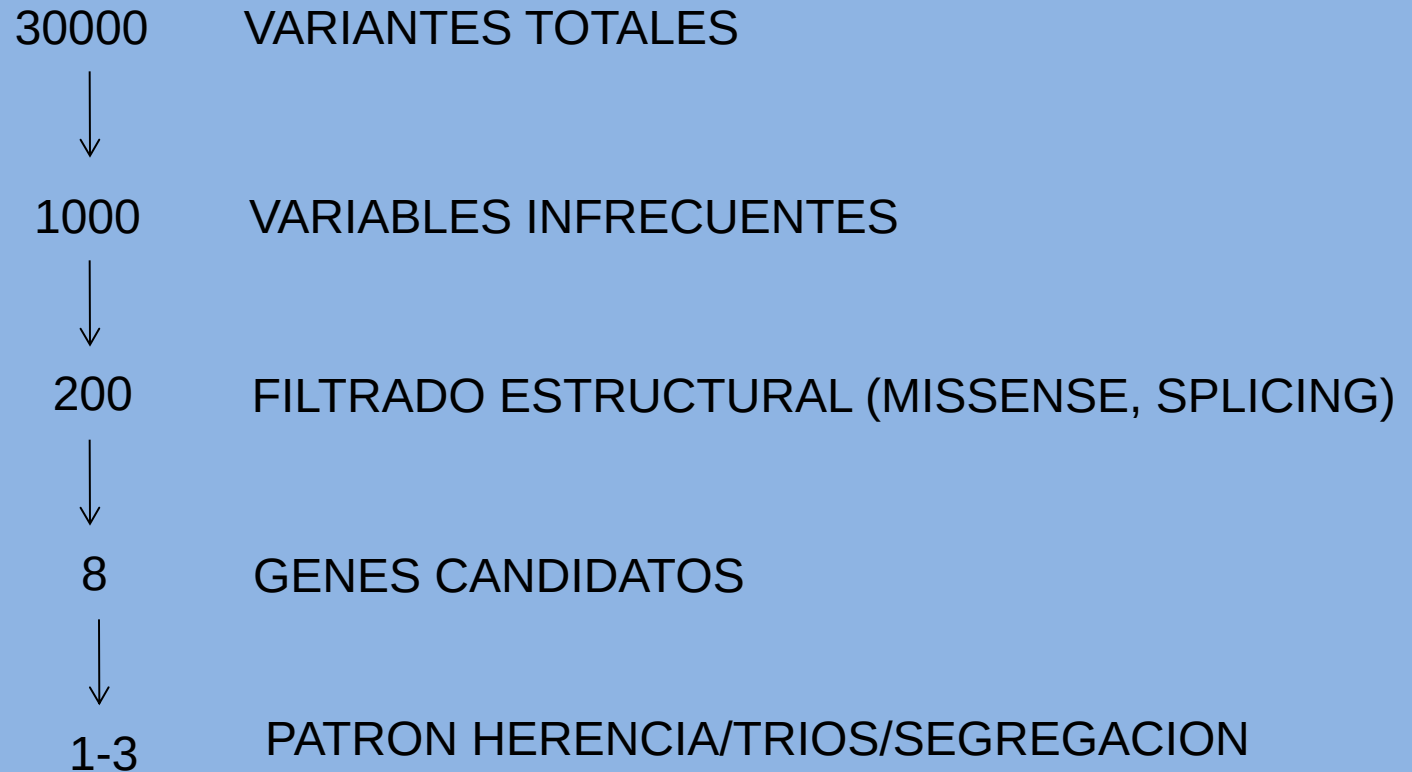
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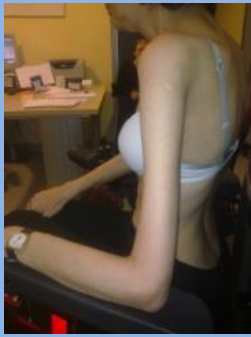
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20 2301308 rs84823 T G . PASS . GT:PL 1/1:10,5,0
....
```

3) ANOTACION (ANNOVAR, KGGSEQ)

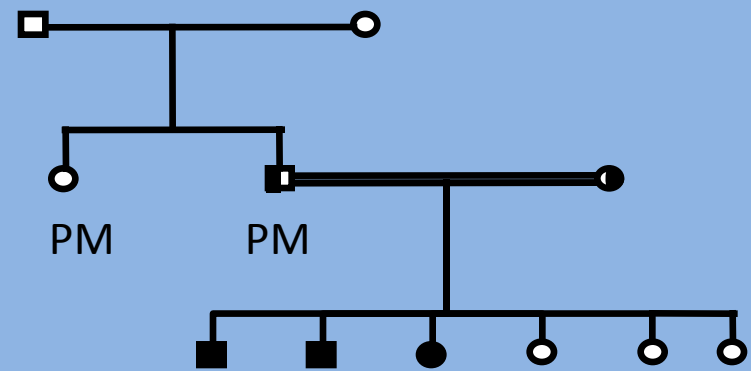
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3	1	1404001	1404001	G	T	UTR3	ATAD3C	NM_001039211:c.*91G>T	.	.	1p36.33	Score=0.90
4	1	5935162	5935162	A	T	splicing	NPHP4	NM_015102:exon22:c.2818-2T>A	.	.	1p36.31	.
5	1	162736463	162736463	C	T	intronic	DDR2	.	.	.	1q23.3	.
6	1	84875173	84875173	C	T	intronic	DNASE2B	.	.	.	1p31.1	.
7	1	13211293	13211294	TC	-	intergenic	HNRNPCP5,PRAMEF3	dist=26967;dist=116902	.	.	1p36.21	Score=0.95
8	1	11403596	11403596	-	AT	intergenic	UBIAD1,PTCHD2	dist=55105;dist=135699	.	.	1p36.22	.
9	1	105492231	105492231	A	ATAAA	intergenic	LOC100129138,NONE	dist=872538;dist=NONE	.	.	1p21.1	.
10	1	67705958	67705958	G	A	exonic	IL23R	.	nonsynonymous SNV	IL23R:NM_1447	1p31.3	.
11	2	234183368	234183368	A	G	exonic	ATG16L1	.	nonsynonymous SNV	ATG16L1:NM_19	2q37.1	.
12	16	50745926	50745926	C	T	exonic	NOD2	.	nonsynonymous SNV	NOD2:NM_0221	16q12.1	.

FILTRADO





3er afectado



PM

1

2015

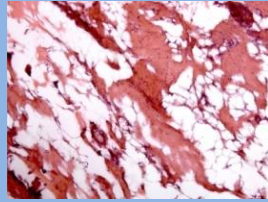
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1995

1985

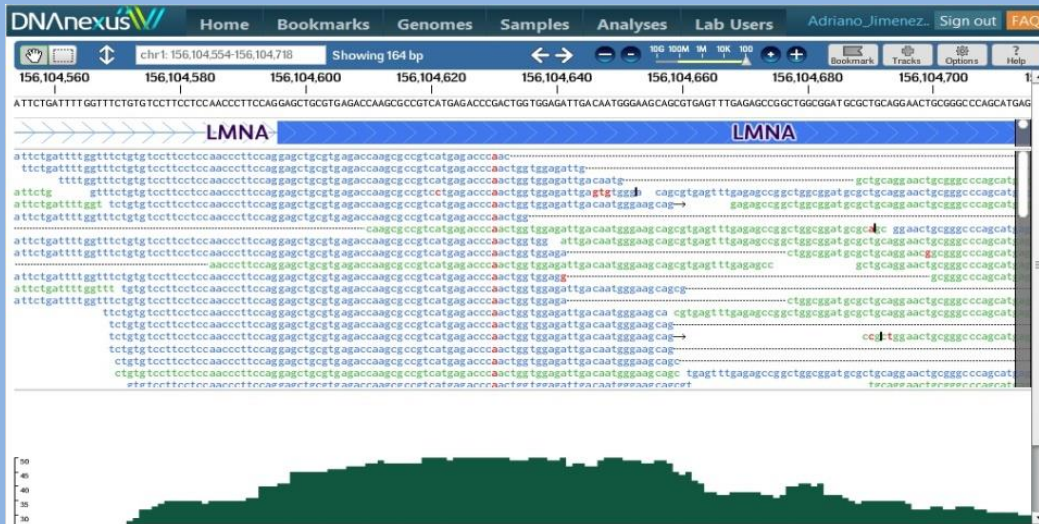
1975

1965



EXOMA

calpaina-3



[illegible]

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Hom	c	c		#####	#####	32	32	32		UBQLN4	NM_020131	CDS	Non-Synony	I
Hom	t	t		#####	#####	4	4	4		UBQLN4	NM_020131	Intron		
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Het	a	g		475.735.998	475.735.998	4	2	2		PAQR6	NM_024897	Intron		
Het	a	g		475.735.998	475.735.998	4	2	2		PAQR6	NM_198406	Intron		
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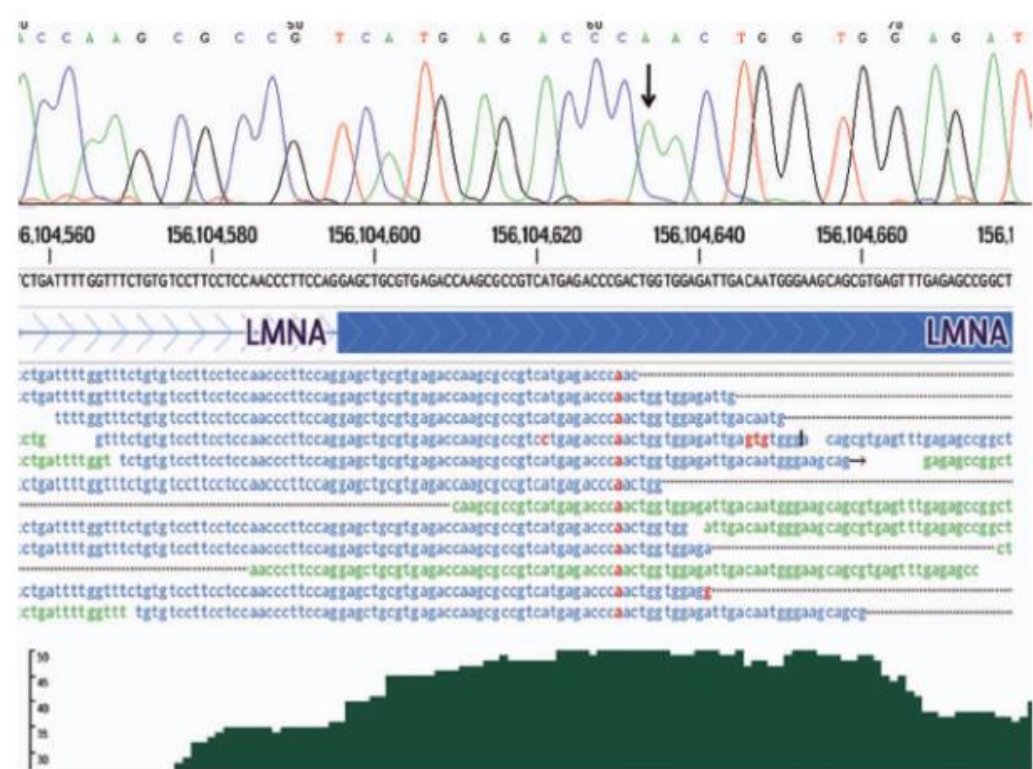
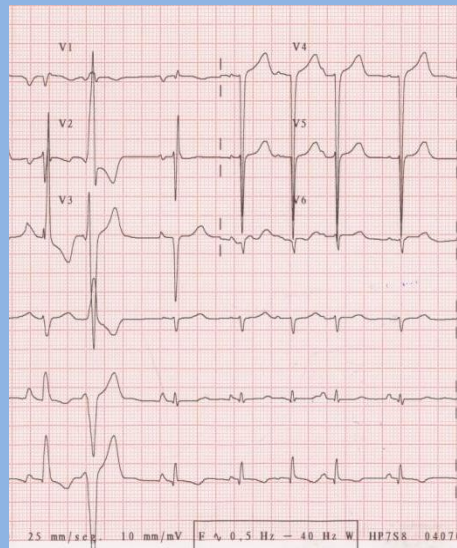
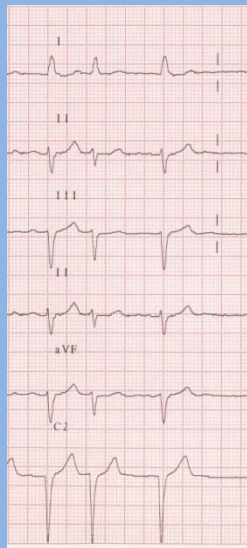
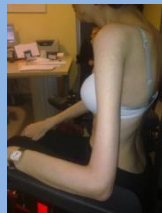
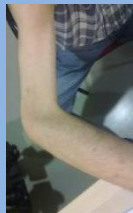
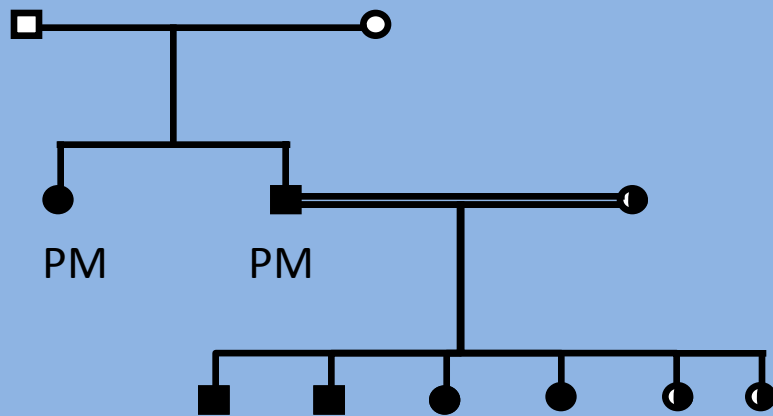


FIGURE 3. Next-generation sequencing multiple alignments at LMNA gene exon 4, showing the G→A mutation; and Sanger sequencing at this locus (top). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CASE OF THE MONTH

AUTOSOMAL RECESSIVE EMERY-DREIFUSS MUSCULAR DYSTROPHY CAUSED BY A NOVEL MUTATION (R225Q) IN THE LAMIN A/C GENE IDENTIFIED BY EXOME SEQUENCING

ADRIANO JIMENEZ-ESCRIG, MD, PhD,^{1,2} ISABEL GOBERNADO, MD,^{2,3} MERCEDES GARCIA-VILLANUEVA, MD, PhD,⁴ and ANTONIO SANCHEZ-HERRANZ, BS, PhD^{2,5}

¹ Servicio de Neurología, Hospital Ramon y Cajal and Universidad de Alcalá, 28034 Madrid, Spain

² Unidad Central de Apoyo a Estudios Genómicos, IRYCIS, Madrid, Spain

³ Servicio de Psiquiatría, Hospital Ramon y Cajal, Madrid, Spain

⁴ Servicio de Anatomía Patológica, Hospital Ramon y Cajal, Madrid, Spain

⁵ Servicio de Neurobiología, Hospital Ramon y Cajal, Madrid, Spain

Accepted 10 October 2011

aje@aje-OptiPlex-990: ~/Public/kggseq

Es 8:50

```
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aje@aje-OptiPlex-990:~/Public/kggseq$ java -Xms256m -Xmx1300m -jar kggseq.jar --no-resource-check --no-lib-check --buildver hg19 --vcf-file /home/aje/Public/men/controlesHC.vcf
--ped-file /home/aje/Public/men/control.ped --db-gene refgene --gene-feature-in 0,1,2,3,4,5,7 --splicing 15 --genotype-filter 1,2 --db-filter hg19_1kg201204,exac --db-score db
nsfp --excel --gene-var-filter 5 --filter-model case-unique --superdup-filter --genome-annot --omim-annot --genes-in ACTA1,AN05,AN05,ADCY6,ATP2A1,ABHD5,ACVR1,CAPN3,CHKB,AG
RN,CACNA1A,ACAD9,AN05,CAV3,COL6A1,ALG2,CACNA1S,ACADL,BAG3,DAG1,COL6A2,ALG14,CAV3,ACADM,BIN1,DES,COL6A3,CHAT,CLCN1,ACADS,C10orf2,DNAJB6,DMD,CHRNA1,HINT1,ACADVL,CAV3,DPM3,DNM2,CH
RNB1,HSPG2,AGL,CCDC78,DYSF,DPM2,CHRNA1,KCNA1,AMPD1,CFL2,FKRP,DYSF,CHRNA1,KCNE3,CPT1B,CNTN1,FKTN,EMD,CHRNA1,KCNJ18,CPT2,COL12A1,GMPPB,FHL1,CNTNAP1,SCN4A,EN03,COL6A1,LMNA,FKRP,COLQ,
ETFA,COL6A2,MYOT,FKTN,DOK7,ETFB,COL6A3,PLEC,GMPPB,DPAAT1,ETFDH,CRYAB,POMGNT1,ISPD,ECEL1,GAA,DES,POMT1,ITGA7,ERBB3,GBE1,DNM2,POMT2,LAMA2,FBN2,GYGI,DYSF,SGCA,LARGE,GFPT1,GYS1,FHL
1,SGCB,LMNA,GLE1,HADH,FKBP14,SGCD,PABPN1,IBA57,HADHA,FLNC,SGCG,POMGNT1,LAMB2,HADHB,GNE,TCAP,POMT1,MUSK,LDHA,HNRNPA1,TNPO3,POMT2,MYBPC1,LPIN1,HNRNPA2B1,TRAPPC11,PTRF,MYH3,PDHA1,
ISCU,TRIM32,SEPN1,MYH8,PFKM,KBTBD13,TTN,SMCHD1,PIEZO2,PGAM2,KLHL40,SYNE1,PIP5K1C,PGK1,KLHL41,SYNE2,PLEC,PGM1,KLHL9,TCAP,PLD2,PHKA1,LAMP2,TMEM43,PREPL,PNPLA2,LDB3,TTN,RAPSN,POL
G2,MATR3,SCN4A,PRKAG2,MEGF10,SYNE1,PUS1,MSTN,TNNI2,PYGM,MTM1,TNNT3,RRM2B,MYBPC1,TPM2,SLC16A1,MYBPC3,ZC4H2,SLC22A5,MYF6,SLC25A20,MYH2,TAZ,MYH7,YARS2,MYH14,MYOT,NEB,OPA1,PABPN1,P
LEC,POLG,POLG2,PTPLA,PUS1,RRM2B,RYR1,SEPN1,SIL1,STAC3,STIM1,SUCLA2,TIA1,TK2,TNNT1,TPM2,TPM3,TRIM32,TTN,VCP,VMA21,YARS2
```

```
--filter-model case-unique
--genes-in ACTA1,AN05,ADCY6,ATP2A1,ABHD5,ACVR1,CAPN3,CHKB,AGRN,CACNA1A,ACAD9,CAV3,COL6A1,ALG2,CACNA1S,ACADL,BAG3,DAG1,COL6A2,ALG14,ACADM,BIN1,DES,COL6A3,CHAT,CLCN1,ACADS,C10orf
2,DNAJB6,DMD,CHRNA1,HINT1,ACADVL,DPM3,DNM2,CHRN1,HSPG2,AGL,CCDC78,DYSF,DPM2,CHRN1,KCNA1,AMPD1,CFL2,FKRP,CHRNA1,KCNE3,CPT1B,CNTN1,FKTN,EMD,CHRNA1,KCNJ18,CPT2,COL12A1,GMPPB,FHL1,C
NTNAP1,SCN4A,EN03,LMNA,COL0,ETFA,MYOT,DOK7,ETFB,PLEC,DPA1,ETFDH,CRYAB,POMGNT1,ISPD,ECEL1,GAA,POMT1,ITGA7,ERBB3,GBE1,POMT2,LAMA2,FBN2,GYGI,SGCA,LARGE,GFPT1,GYS1,SGCB,GLE1,HADH
,FKBP14,SGCD,PABPN1,IBA57,HADHA,FLNC,SGCG,LAMB2,HADHB,GNE,TCAP,MUSK,LDHA,HNRNP1,TNP03,MYBPC1,LPIN1,HNRNP2B1,TRAPPC11,PTRF,MYH3,PDHA1,ISCU,TRIM32,SEPN1,MYH8,PFKM,KBTBD13,TTN,S
MCHD1,PIEZO2,PGAM2,KLHL40,SYNE1,PIP5K1C,PGK1,KLHL41,SYNE2,PGM1,KLHL9,PLD2,PHKA1,LAMP2,TMEM43,PREPL,PNPLA2,LDB3,RAPSN,POLG2,MATR3,PRKAG2,MEGF10,PUS1,MSTN,TNNI2,PYGM,MTM1,TNNT3,
RRM2B,TPM2,SLC16A1,MYBPC3,ZC4H2,SLC22A5,MYF6,SLC25A20,MYH2,TAZ,MYH7,YARS2,MYH14,NEB,OPA1,POLG,PTPLA,RYR1,SIL1,STAC3,STIM1,SUCLA2,TIA1,TK2,TNNT1,TPM3,VCP,VMA21
---genome-annot
--omim-annot
--superdup-filter
--db-score dbnsfp
--mendel-causing-predict
INFO 2015-03-25 08:50:53 - Encoding pedigree file /home/aje/Public/men/control.ped ...
INFO 2015-03-25 08:50:53 - Reading variants in /home/aje/Public/men/controlesHC.vcf
INFO 2015-03-25 08:51:04 -
6867635 missing genotypes are ignored
6893 genotypes are ignored due to low depth at the site with a genotype <4
142477 genotypes are ignored due to low quality of specific genotyping quality <10.0
37344 genotypes are ignored because the fraction of the reads carrying alternative allele >= 0.05 at a reference-allele homozygous genotype and that is <= 0.25 at a heterozygous
s genotype and that is <= 0.75 at an alternative-allele homozygous genotype
114708 genotypes are ignored because their second smallest Phred-scaled likelihoods (PL) are < 20.
299723 variants are ignored due to low sequencing quality (<50.0)
765214 variant-lines (76493 indels) are scanned; and 465491 variants of 17 individual(s) are valid in /home/aje/Public/men/controlesHC.vcf
INFO 2015-03-25 08:51:04 - 20041 variants are ignored by genotype-based hard-filtering;
445450 variant(s) are left after filtration according to inheritance mode at genotypes, 1,2!
INFO 2015-03-25 08:51:04 - 66 variant(s) are left after filtration according to case-unique!
Parsed variants INFO 2015-03-25 08:51:25 - 43 variant(s) exist in lkg201204, which contains 39706714 effective variants.
Parsed variants INFO 2015-03-25 08:51:30 - 35 variant(s) exist in exac, which contains 10205998 effective variants.
INFO 2015-03-25 08:51:30 - 26 variant(s) with minor allele frequency [0, 0.01) in the reference datasets above are left!
INFO 2015-03-25 08:51:33 - 52259 transcripts of 25953 genes have been read in the dataset kggseq_hg19_refGene.txt.gz!
INFO 2015-03-25 08:51:34 -
0.frameshift: 0 (0%)
1.nonframeshift: 0 (0%)
2.stoploss: 0 (0%)
3.stopgain: 0 (0%)
4.missense: 7 (26.923%)
5.splicing: 0 (0%)
6.synonymous: 1 (3.846%)
7.exonic: 0 (0%)
8.5UTR: 0 (0%)
9.3UTR: 8 (30.769%)
10.intronic: 3 (11.538%)
11.upstream: 0 (0%)
12.downstream: 0 (0%)
13.ncRNA: 0 (0%)
14.intergenic: 0 (0%)
15.monomorphic: 0 (0%)
16.unknown: 7 (26.923%)
INFO 2015-03-25 08:51:34 - 7 variant(s) are left after filtering by gene features.
INFO 2015-03-25 08:51:34 - 1 variant(s) are left after filtering those outside specified genes!
INFO 2015-03-25 08:51:34 - 1 sequence variant(s) are highlighted by OMIM information!
INFO 2015-03-25 08:51:35 - 41160 reference super duplicate regions have been read!
INFO 2015-03-25 08:51:35 - 1 variant(s) are left after filtering those in super-duplicated regions registered in a data set genomicSuperDups table of UCSC!
Parsed variants
Parsed variants ords
Parsed variants INFO 2015-03-25 08:51:47 - 1 coding nonsynonymous variants among 1 are assigned functional prediction scores.
INFO 2015-03-25 08:51:47 - The number of predicted disease-causal and non-disease-causal variants are 1(#gene 1) and 0 among 1 variants on the genome according to the Logistic
regression prediction model trained by ExoVar dataset (http://statgenpro.psychiatry.hku.hk/limx/kggseq/download/ExoVar.xls)
INFO 2015-03-25 08:51:50 - Final variants are saved in /home/aje/Public/kggseq/kggseq.flt.xlsx with Excel format.
INFO 2015-03-25 08:51:50 - 1 sequence variant(s) are left finally!
INFO 2015-03-25 08:51:50 - Elapsed time: 0 min. 56 sec.
The log information is save in ./kggseq.log
aje@aje-OptiPlex-990:~/Public/kggseq$
```

$$f(x) \Sigma =$$

R2

R2

Emery-Dreifuss muscular dystrophy 3, AR, 181350 (3)|Heart-hand syndrome, Slovenian type, 610140 (3)|Hutchinson-Gilford progeria, 176670 (3)|Lipodystrophy, familial partial, 2, 151660 (3)

Emery-Dreifuss muscular dystrophy 3, AR, 181350 (3)|Heart-hand syndrome, Slovenian type, 610140 (3)|Hutchinson-Gilford progeria, 176670 (3)|Lipodystrophy, familial partial, 2, 151660 (3)

aje@aje-OptiPlex-990: ~/Public/kggseq

To specify path and prefix name of outputs, add the '--out your/path' option; it is ./kggseq by default

INFO 2015-03-25 08:31:51 -

```
@-----@
|      KGGSeq      |   v0.7   |  18/Jan./2015  |
|-----|-----|
| (C) 2011 Miao Xin Li,  limx54@yahoo.com  |
|-----|-----|
| For documentation, citation & bug-report instructions: |
| http://statgenpro.psychiatry.hku.hk/kggseq             |
|-----|-----|
@-----@
```

Effective settings :

--buildver hg19

--vcf-file /home/aje/Public/men/controlesHC.vcf

--ped-file /home/aje/Public/men/control.ped

--excel

--genotype-filter 2,3,4

--db-gene refgene

--gene-feature-in 0,1,2,3,4,5,7

--splicing 15

--db-filter lkg201204,exac

--filter-model case-unique

--genes-in ACTA1, ANO5, ADCY6, ATP2A1, ABHD5, ACVR1, CAPN3, CHKB, AGRN, CACNA1A, ACAD9, CAV3, COL6A1, ALG2, CACNA1S, ACADL, BAG3, DAG1, COL6A2, ALG14, ACADM, BIN1, DES, COL6A3, CHAT, CLCN1, ACADS, C10orf2, DNAJB6, DMD, CHRNA1, HINT1, ACADVL, DPM3, DNMT2, CHRNA1, HSPG2, AGL, CCDC78, DYSF, DPM2, CHRNA1, AMPD1, CFL2, FKBP, CHRNE, KCNE3, CPT1B, CNTN1, FKTN, EMD, CHRNA1, KCNJ18, CPT2, COL12A1, GMPBP, FHL1, C, NTNAP1, SCN4A, ENO3, LMNA, COLQ, ETFA, MYOT, DOK7, ETFB, PLEC, DPAGT1, ETFDH, CRYAB, POMGNT1, ISPD, ECEL1, GAA, POMT1, ITGA7, ERBB3, GBE1, POMT2, LAMA2, FBN2, GYG1, SGCA, LARGE, GFPT1, GYS1, SGCB, GLE1, HADH, FKBP14, SGCD, PABPN1, IBA57, HADHA, FLNC, SGCG, LAMB2, HADHB, GNE, TCAP, MUSK, LDHA, HNRNPA1, TNP03, MYBPC1, LPIN1, HNRNPA2B1, TRAPPC11, PTRF, MYH3, PDHA1, ISCU, TRIM32, SEP1, MYH8, PFKM, KBTBD13, TTN, S, MCHD1, PIEZO2, PGAM2, KLHL40, SYNE1, PIP5K1C, PGK1, KLHL41, SYNE2, PGM1, KLHL9, PLOD2, PHKA1, LAMP2, TMEM43, PREPL, PNPLA2, LDB3, RAPS, POLG2, MATR3, PRKAG2, MEGF10, PUS1, MSTN, TNNT1, PYGM, MTM1, TNNT3, RRM2B, TPM2, SLC16A1, MYBPC3, ZC4H2, SLC22A5, MYF6, SLC25A20, MYH2, TAZ, MYH7, YARS2, MYH14, NEB, OPA1, POLG, PTPLA, RYR1, SIL1, STAC3, STIM1, SUCLA2, TIA1, TK2, TNNT1, TPM3, VCP, VMA21

---genome-annot

--omim-annot

--superdup-filter

--db-score dbnsfp

--mendel-causing-predict

INFO 2015-03-25 08:31:51 - Encoding pedigree file /home/aje/Public/men/control.ped ...

INFO 2015-03-25 08:31:51 - Reading variants in /home/aje/Public/men/controlesHC.vcf

INFO 2015-03-25 08:32:02 -

6867635 missing genotypes are ignored

6893 genotypes are ignored due to low depth at the site with a genotype <4

142477 genotypes are ignored due to low quality of specific genotyping quality <10.0

37344 genotypes are ignored because the fraction of the reads carrying alternative allele ≥ 0.05 at a reference-allele homozygous genotype and that is ≤ 0.25 at a heterozygous genotype and that is ≤ 0.75 at an alternative-allele homozygous genotype

114708 genotypes are ignored because their second smallest Phred-scaled likelihoods (PL) are < 20.

299723 variants are ignored due to low sequencing quality (<50.0)

765214 variant-lines (76493 indels) are scanned; and 465491 variants of 17 individual(s) are valid in /home/aje/Public/men/controlesHC.vcf

INFO 2015-03-25 08:32:03 - 37742 variants are ignored by genotype-based hard-filtering;

427749 variant(s) are left after filtration according to inheritance mode at genotypes, 2,3,4!

INFO 2015-03-25 08:32:03 - 4230 variant(s) are left after filtration according to case-unique!

Parsed variants INFO 2015-03-25 08:32:25 - 3216 variant(s) exist in lkg201204, which contains 39706714 effective variants.

Parsed variants INFO 2015-03-25 08:32:31 - 1484 variant(s) exist in exac, which contains 10205998 effective variants.

INFO 2015-03-25 08:32:31 - 1457 variant(s) with minor allele frequency [0, 0.01] in the reference datasets above are left!

INFO 2015-03-25 08:32:34 - 52259 transcripts of 25953 genes have been read in the dataset kggseq_hg19_refGene.txt.gz!

INFO 2015-03-25 08:32:35 -

0.frameshift: 11 (0.755%)

1.nonframeshift: 12 (0.824%)

2.stoploss: 0 (0%)

3.stopgain: 5 (0.343%)

4.missense: 306 (21.002%)

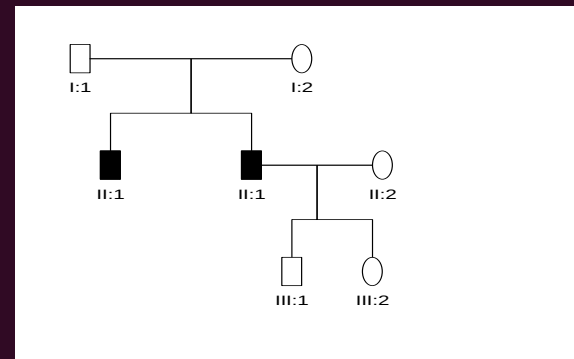
5.splicing: 2 (0.137%)

6.synonymous: 203 (13.933%)

7.exonic: 0 (0%)

8.5UTR: 132 (9.06%)

9.3UTR: 626 (42.965%)



```

--filter-model case-unique
--genes-in ACTA1,AN05,ADCY6,ATP2A1,ABHD5,ACVR1,CAPN3,CHKB,AGRN,CACNA1A,ACAD9,CAV3,COL6A1,ALG2,CACNA1S,ACADL,BAG3,DAG1,COL6A2,ALG14,ACADM,BIN1,DES,COL6A3,CHAT,CLCN1,ACADS,C10orf
2,DNAJB6,DMD,CHRNA1,HINT1,ACADVL,DPM3,DNM2,CHRN1,HSPG2,AGL,CCDC78,DYSF,DPM2,CHRNA1,AMPD1,CFL2,FKRP,CHRNE,KCNE3,CPT1B,CNTN1,FKTN,EMD,CHRNA1,KCNJ18,CPT2,COL12A1,GMPPB,FHL1,C
NTNAP1,SCN4A,EN03,LMNA,COLO,ETFA,MYOT,DOK7,ETFB,PLEC,DPAAT1,ETFDH,CRYAB,POMGNT1,ISPD,ECEL1,GAA,POMT1,ITGA7,ERBB3,GBE1,POMT2,LAMA2,FBN2,GYGI,SGCA,LARGE,GFPT1,GYS1,SGCB,GLE1,HADH
,FKBP14,SGCD,PABPN1,IBA57,HADHA,FLNC,SGCG,LAMB2,HADHB,GNE,TCAP,MUSK,LDHA,HNRNPA1,TNPO3,MYBPC1,LPIN1,HNRNPA2B1,TRAPPC11,PTRF,MYH3,PDHA1,ISCU,TRIM32,SEPN1,MYH8,PFKM,KBTBD13,TTN,S
MCHD1,PIEZ02,PGAM2,KLHL40,SYNE1,PIP5K1C,PGK1,KLHL41,SYNE2,PGM1,KLHL9,PLD2,PHKA1,LAMP2,TMEM43,PREPL,PNPLA2,LDB3,RAPSN,POLG2,MATR3,PRKAG2,MEGF10,PUS1,MSTN,TNNI2,PYGM,MTM1,TNNT3,
RBM2B,TPM2,SLC16A1,MYBPC3,ZC4H2,SLC22A5,MYF6,SLC25A20,MYH2,TAZ,MYH7,YARS2,MYH14,NEB,OPA1,POLG,PTPLA,RYR1,SIL1,STAC3,STIM1,SUCLA2,TIA1,TK2,TNNT1,TPM3,VCP,VMA21
---genome-annot
--omim-annot
--superdup-filter
--db-score dbnsfp
--mendel-causing-predict
INFO 2015-03-25 08:42:45 - Encoding pedigree file /home/aje/Public/men/control.ped ...
INFO 2015-03-25 08:42:45 - Reading variants in /home/aje/Public/men/controlesHC.vcf
INFO 2015-03-25 08:42:56 -
6867635 missing genotypes are ignored
6893 genotypes are ignored due to low depth at the site with a genotype <4
142477 genotypes are ignored due to low quality of specific genotyping quality <10.0
37344 genotypes are ignored because the fraction of the reads carrying alternative allele >= 0.05 at a reference-allele homozygous genotype and that is <= 0.25 at a heterozygous
genotype and that is <= 0.75 at an alternative-allele homozygous genotype
114708 genotypes are ignored because their second smallest Phred-scaled likelihoods (PL) are < 20.
299723 variants are ignored due to low sequencing quality (<50.0)
765214 variant-lines (76493 indels) are scanned; and 465491 variants of 17 individual(s) are valid in /home/aje/Public/men/controlesHC.vcf
INFO 2015-03-25 08:42:56 - 42097 variants are ignored by genotype-based hard-filtering;
423394 variant(s) are left after filtration according to inheritance mode at genotypes, 2,3,4!
INFO 2015-03-25 08:42:56 - 4863 variant(s) are left after filtration according to case-unique!
Parsed variants INFO 2015-03-25 08:43:17 - 3483 variant(s) exist in lkg201204, which contains 39706714 effective variants.
Parsed variants INFO 2015-03-25 08:43:23 - 1650 variant(s) exist in exac, which contains 10205998 effective variants.
INFO 2015-03-25 08:43:23 - 1753 variant(s) with minor allele frequency [0, 0.01] in the reference datasets above are left!
INFO 2015-03-25 08:43:26 - 52259 transcripts of 25953 genes have been read in the dataset kggseq_hg19_refGene.txt.gz!
INFO 2015-03-25 08:43:27 -
0.frameshift: 10 (0.57%)
1.nonframeshift: 20 (1.141%)
2.stoploss: 0 (0%)
3.stopgain: 8 (0.456%)
4.missense: 366 (20.878%)
5.splicing: 9 (0.513%)
6.synonymous: 208 (11.865%)
7.exonic: 0 (0%)
8.5UTR: 134 (7.644%)
9.3UTR: 704 (40.16%)
10.intronic: 61 (3.48%)
11.upstream: 18 (1.027%)
12.downstream: 11 (0.627%)
13.ncRNA: 173 (9.869%)
14.intergenic: 26 (1.483%)
15.monomorphic: 0 (0%)
16.unknown: 5 (0.285%)
INFO 2015-03-25 08:43:27 - 413 variant(s) are left after filtering by gene features.
INFO 2015-03-25 08:43:27 - 10 variant(s) are left after filtering those outside specified genes!
INFO 2015-03-25 08:43:28 - 8 sequence variant(s) are highlighted by OMIM information!
INFO 2015-03-25 08:43:28 - 41160 reference super duplicate regions have been read!
INFO 2015-03-25 08:43:28 - 10 variant(s) are left after filtering those in super-duplicated regions registered in a data set genomicSuperDups table of UCSC!
Parsed variants
Parsed variants ords
Parsed variants INFO 2015-03-25 08:43:51 - 9 coding nonsynonymous variants among 10 are assigned functional prediction scores.
INFO 2015-03-25 08:43:52 - The number of predicted disease-causal and non-disease-causal variants are 9(gene 7) and 0 among 10 variants on the genome according to the Logisti
c regression prediction model trained by ExoVar dataset (http://statgenpro.psychiatry.hku.hk/limx/kggseq/download/ExoVar.xls)
INFO 2015-03-25 08:43:54 - Final variants are saved in /home/aje/Public/kggseq/kggseq.flt.xlsx with Excel format.
INFO 2015-03-25 08:43:54 - 10 sequence variant(s) are left finally!
INFO 2015-03-25 08:43:54 - Elapsed time: 1 min. 8 sec.
The log information is save in ./kggseq.log
aje@aje-OptiPlex-990:~/Public/kggseq$

```

	A	B	C	D	E	F	G	H	I
1	Chromosome	StartPosition	onceAlternative	ImportantFeature	ImportantGeneFe	RefGeneFeatures	SIFT_score	typhen2_HDIV_sc	typhen2_HVA
2	1	983443	G/A	AGRN	missense	576(36Exons):exon23:c.G3803A:p.G12	1	0.003	0.0060000
3	1	95538372	G/T	ALG14	missense	144988(4Exons):exon1:c.C83A:p.S28	0.1800000072	0.9470000267	0.2540000
4	1	95538424	C/T	ALG14	missense	144988(4Exons):exon1:c.G31A:p.A11	0.400000006	0.0160000008	0.0020000
5	2	179517605	G/A	TTN	missense	2Exons):intronic47;TTN:NM_00125685	.	.	.
6	2	238253403	G/A	COL6A3	missense	7166(41Exons):exon33:c.C5437T:p.R1	0.0399999991	0.9980000257	0.8169999
7	3	48916844	C/T	SLC25A20	missense	1000387(9Exons):exon4:c.G364A:p.V	0.9599999785	0.3689999878	0.07900000
8	17	78085800	T/C	GAA	missense	079803(21Exons):exon13:c.T1655C:p	0	1	0.9950000
9	19	3651960	G/A	PIP5K1C	missense	012398(18Exons):exon8:c.C991T:p.R3	0	0.9990000129	0.8270000
10	19	38973933	A/G	RYR1	missense	1571V&missense;RYR1:NM_00104272	0.9300000072	0.9909999967	0.9779999
11	19	39009932	G/A	RYR1	missense	9366H&missense;RYR1:NM_00104272	0.0099999998	0.8899999857	0.2759999
12	19	39034191	A/G	RYR1	missense	9933C&missense;RYR1:NM_00104272	0	1	0.9969999
13	21	47552005	C/T	COL6A2	missense	1849(28Exons):exon28:c.C2599T:p.R8	0.0199999996	1	0.9869999

J	K	L	M	N	O	P	Q
<u>LRT_score</u>	<u>altFreq@1kg20120</u>	<u>altFreq@exac</u>	<u>totFeatureForRe</u>	<u>GeneDescription</u>	<u>Pseudogenes</u>	<u>DiseaseName(s)MIMid</u>	<u>GeneMIMid</u>
0.9083529711	N	0.0001985	.	agrin (Approved)	.	Myasthenia, limb-girdle, familial, 254300 (3)	103320
0.3723140061	0.0005	0.0001057	.	glucosaminyl transfer	.	.	.
0.4576810002	0.0018	0.006937	.	glucosaminyl transfer	.	.	.
.	0.0027	0.0063899998	.	titin (Approved)	.	608807 (3) Myopathy, early-onset, with fatal cardiomyopathy	188840
0.0035570001	0.0009	0.0006751	.	type VI, alpha 3 (A	.	myopathy, 158810 (3) Ulrich congenital muscular dystrophy, 2	120250
0.0040569999	0.0005	0.0001626	.	e/acylcarnitine trans	SLC25A20P1	Carnitine-acylcarnitine translocase deficiency, 212138 (3)	613698
0.000001	N	0.00001626	.	lase, alpha; acid (Ap	.	Glycogen storage disease II, 232300 (3)	606800
0.000056	0.0005	0.0007899	.	osphate 5-kinase, ty	.	Lethal congenital contractural syndrome 3, 611369 (3)	606102
0.000002	0.0041	0.0006042	.	receptor 1 (skeletal)	.	gia, 255320 (3) Neuromuscular disease, congenital, with unif	180901
0.001052	0.0037	0.0009108	.	receptor 1 (skeletal)	.	gia, 255320 (3) Neuromuscular disease, congenital, with unif	180901
0.000014	0.0037	0.0009189	.	receptor 1 (skeletal)	.	gia, 255320 (3) Neuromuscular disease, congenital, with unif	180901
0.0385139994	N	0.0009595	.	type VI, alpha 2 (A	.	(3) Myosclerosis, congenital, 255600 (3) Ulrich congenital mu	120240

RYR1 Mutations Are a Common Cause of Congenital Myopathies with Central Nuclei

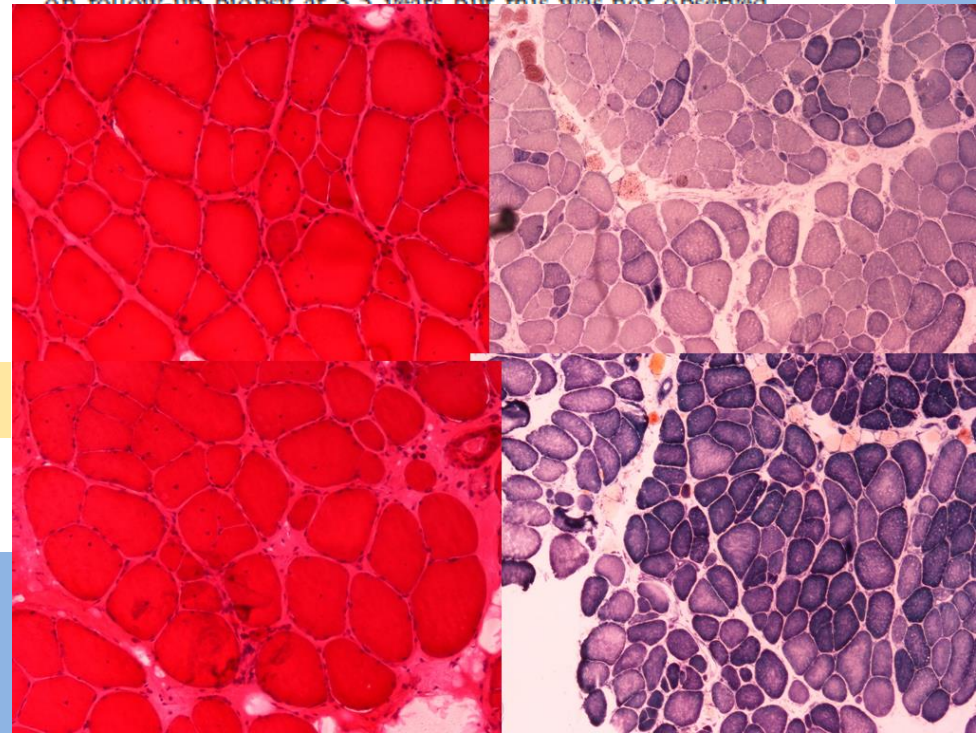
J.M. Wilmshurst, MD,¹ S. Lillis, BSc,² H. Zhou, PhD,³ K. Pillay, MBChB,⁴ H. Henderson, PhD,⁵ W. Kress, PhD,⁶ C.R. Müller, PhD,⁶ A. Ndong, MBBS,¹ V. Cloke, BSc,² T. Cullup, BSc,² E. Bertini, MD,⁷ C. Boennemann, PhD,⁸ V. Straub, PhD,⁹ R. Quinlivan, MD,¹⁰ J.J. Dowling, PhD,¹¹ S. Al-Sarraj, MD,¹² S. Treves, PhD,¹³ S. Abbs, PhD,² A.Y. Manzur, MBBS,³ C.A. Sewry, PhD,^{3,10} F. Muntoni, MD,³ and H. Jungbluth, MD, PhD^{14,15}

themia (MH) reactions were recorded but none of the patients had been formally assessed by in vitro contracture testing (IVCT).

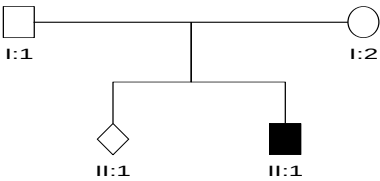
Muscle Histology

We reviewed all available muscle biopsies (Table 2; Fig 2). First muscle biopsies were performed between 2 months and 5 years of age (median age 10.5 months). On the first muscle biopsy, a substantial increase in fibers with nuclear centralization (median 11%; range 3–30%) and with nuclear internalization (median 10.5%; range 3–34%), often multiple, was present in all patients (Fig 2A). Fibers containing central nuclei were often very small. Mild to moderate increases in fat and/or connective tissues were seen in most cases. In one patient (Patient 17) necklace fibers¹⁹ were identified as an additional finding. Type 1 predominance (Fig 2B,C) was present in the first muscle biopsy in all patients except Patients 9 and 10. In addition, type 2 hy-

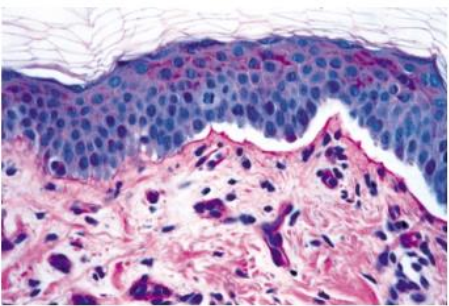
perrophy and fiber type disproportion with type 2 fibers at least 25% larger than type 1 fibers (Fig 3A–C) was present in 12 biopsies. With stains for oxidative enzymes central accumulation of stain (Fig 2C) was consistent but peripheral halos commonly seen in X-linked myotubular myopathy were only seen in three patients (Patients 5, 14, 17). Radial strands were not observed. None of our patients had core-like structures on the first muscle biopsy but two had a “moth-eaten” appearance with oxidative enzyme stains (Patients 2, 6). Central or minicores developed with age in two out of three patients who had follow-up biopsies later in life (Patients 16, 17), but were not a prominent feature. In addition, Z-line streaming was observed on 14 biopsies where electron microscopy was performed and on five of these additional core-like structures were seen (Fig 2D). One patient had unevenness of stain with ATPase reactions on follow-up biopsy at 3.5 years but this was not observed



HIPERCKEMIA



G8	$f(x) \sum =$	PLEC:NM_000445(33Exons):exon33:c.G7903A:p.A2635T&missense;PLEC:NM_201378(32Exons):exon						
	A	B	C	D	E	F	G	
1	Chromosome	StartPosition	anceAlternative	rsID	ImportantFeatur	ImportantGeneFe	RefGeneFeatures	e
2	2	44586902	G/A	.	PREPL	splicing	NM_001171613(14E	
3	2	152381033	C/G	.	NEB	missense	&missense; NEB:NM	
4	2	179417104	T/C	.	TTN	missense	M_133432(192Exons	
5	2	179596554	T/C	.	TTN	missense	13316G:p.Y4439C&	
6	2	233396103	C/G	.	CHRND	missense	557(11Exons):exon7	
7	6	129618997	A/T	.	LAMA2	missense	&missense; LAMA2	
8	8	144996167	C/T	.	PLEC	missense	missense; PLEC:NM	
9	8	145024570	C/A	.	PLEC	missense	EC:NM_201379(32E	
10	16	28893875	G/A	.	ATP2A1	missense	9Exons):exon5:c.G4	
11	19	47259734	G/C	.	FKRP	missense	issense; FKRP:NM	



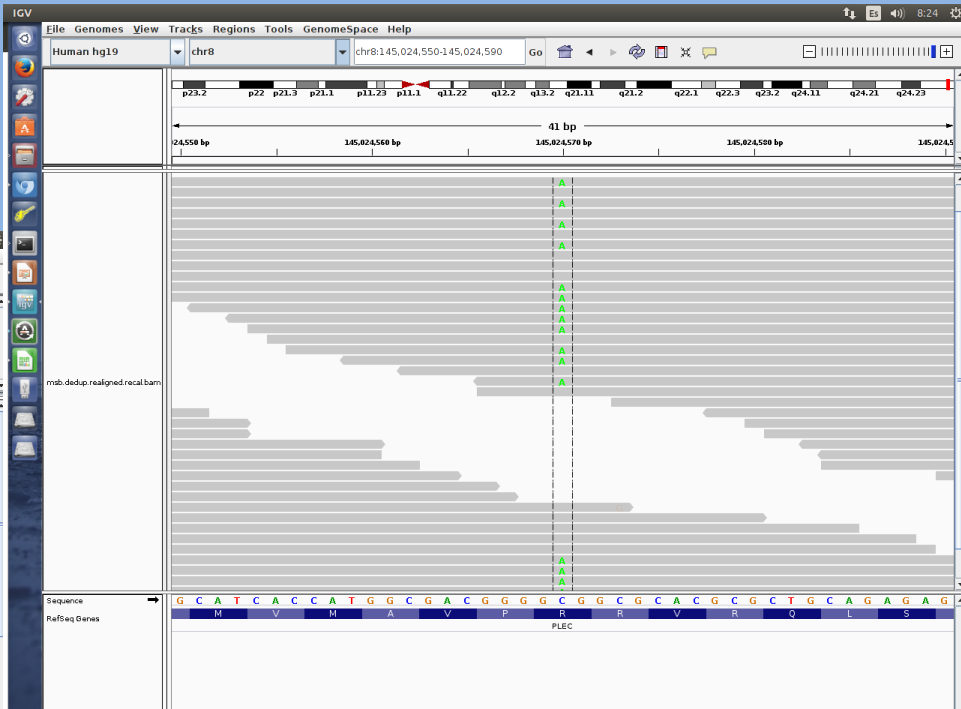
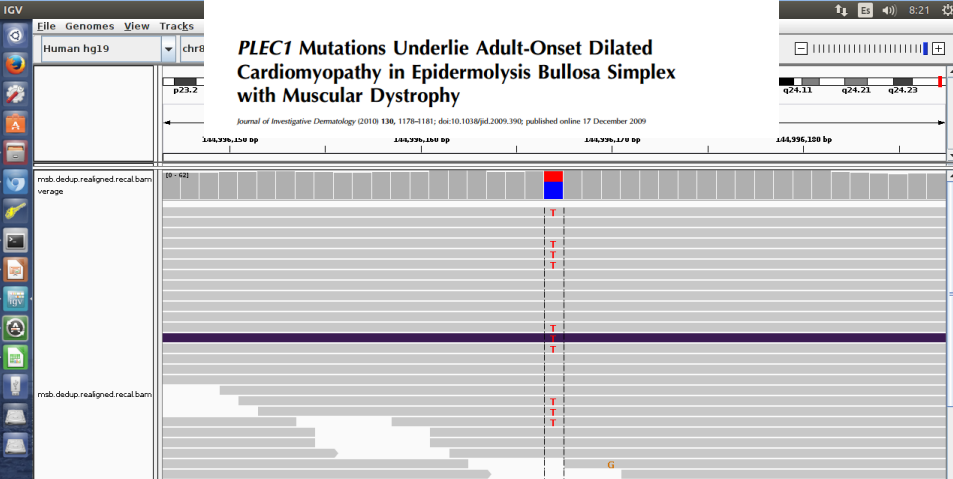
Epidermolysis Bullosa: Novel and *De Novo* Premature Termination Codon and Deletion Mutations in the Plectin Gene Predict Late-Onset Muscular Dystrophy

Fatima Rouan, Leena Pulkkinen, Guerin Meneguzzi,* Sal LaForgia, Patrice Hyde, Dae Un Kim,† Gabriele Richard, and Joumi Uitto
Departments of Dermatology and Cutaneous Biology, and Biochemistry and Molecular Pharmacology, and Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Jefferson Medical College, Philadelphia, Pennsylvania, U.S.A., *U3M5 INSERM, Faculté de Médecine, Nice, France; †Department of Pathology, St. Barnabas Medical Center, Livingston, New Jersey, U.S.A.

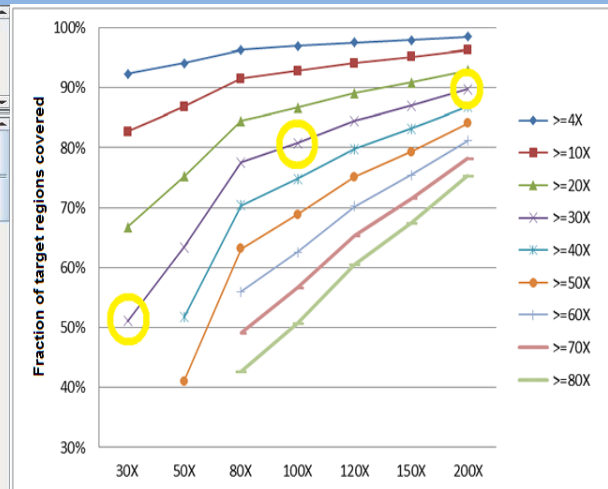
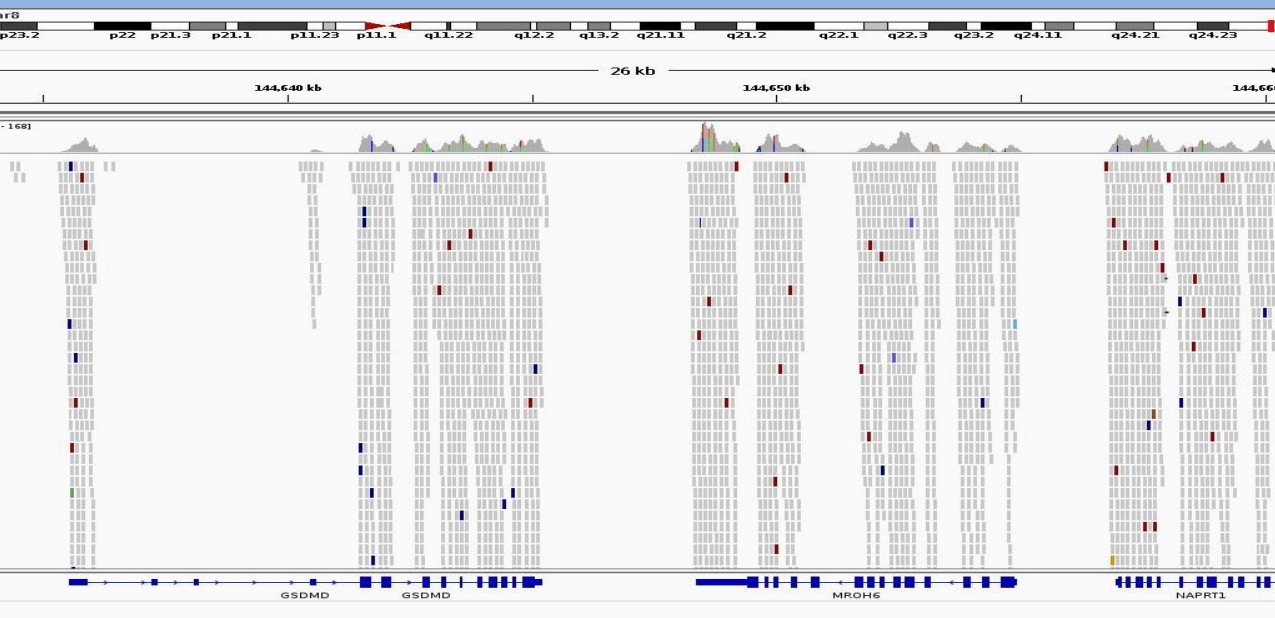
LETTERS TO THE EDITOR

PLEC1 Mutations Underlie Adult-Onset Dilated Cardiomyopathy in Epidermolysis Bullosa Simplex with Muscular Dystrophy

Journal of Investigative Dermatology (2010) 130, 1178–1181; doi:10.1038/jid.2009.390; published online 17 December 2009

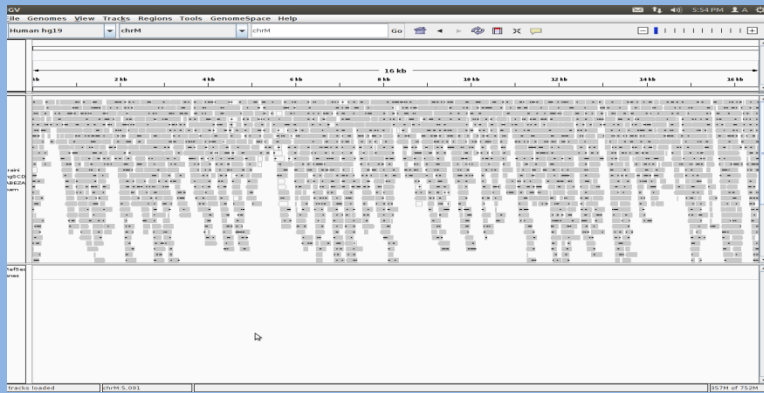


COBERTURA Y TIPOS DE EXOMA



TIPO DE ESTUDIO	MARCA COMERCIAL	VERSION	COBERTURA	Nº GENES	COSTE
EXOMA	AGILENT	V2,V3,V4,V5	30-50-80-100-150	27.500	600-1000
	NIMBLEGENE	V2,V3			
	ILLUMINA	TRUESEQ, NEXTERA			
EXOMA CLINICO	ILLUMINA	TRUESEQONE	150	4875	600
	AGILENT	SURESELECT	150	5200	600
PANELES DE GENES	-		200-400	20-100	600

Estudio de genética mitocondrial con NGS



Enfermedades mitocondriales:

- mutaciones puntuales
- deleciones, duplicaciones

Trastornos mitocondriales

S. Fatiga crónica

HiperCKemia

Intolerancia ejercicio

OPEN ACCESS Freely available online

PLOS COMPUTATIONAL BIOLOGY

Next-Generation Sequencing of Human Mitochondrial Reference Genomes Uncovers High Heteroplasmy Frequency

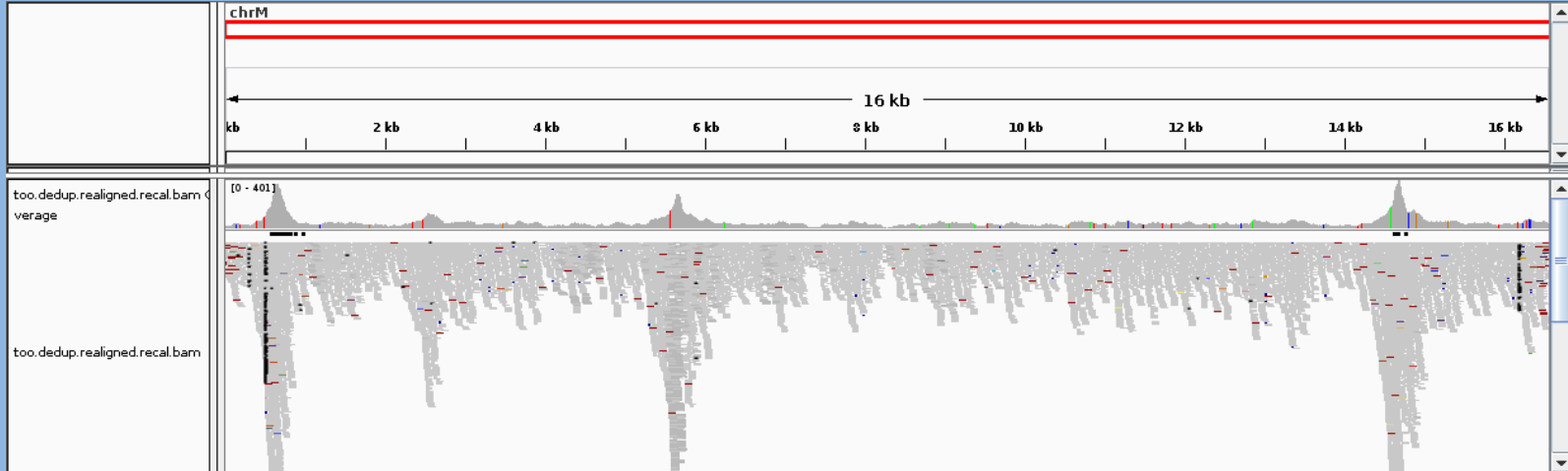
Maria Ximena Sosa^{1,2}, I. K. Ashok Sivakumar^{1,2,3}, Samantha Maragh^{1,3}, Vamsi Veeramachaneni⁴, Ramesh Hariharan⁴, Minothi Parulekar⁴, Karin M. Fredrikson⁵, Timothy T. Harkins^{5,6}, Jeffrey Lin², Andrew B. Feldman², Pramila Tata⁴, Georg B. Ehret¹, Aravinda Chakravarti^{1*}

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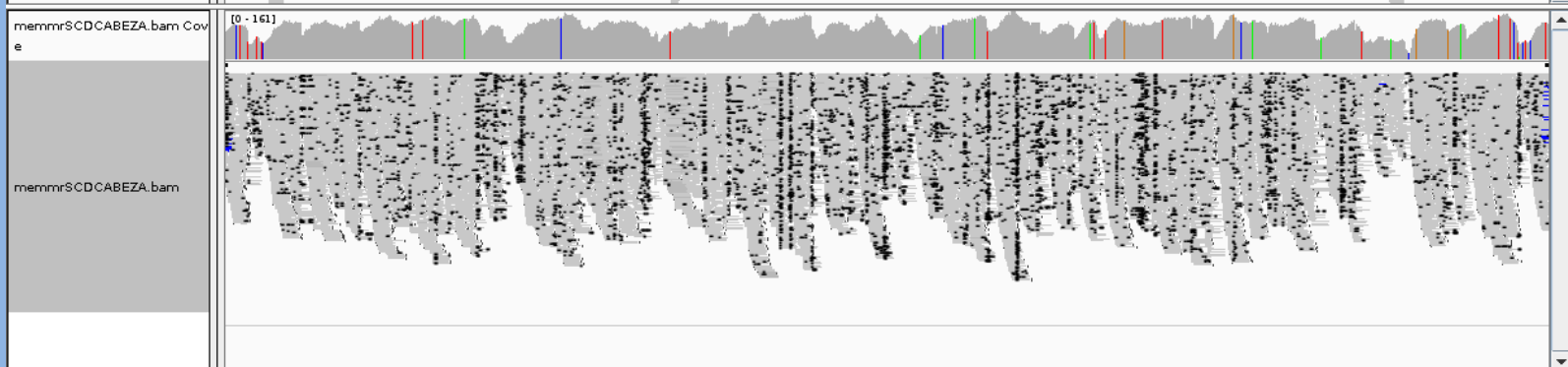
Abstract

We describe methods for rapid sequencing of the entire human mitochondrial genome (mtgenome), which involve long-range PCR for specific amplification of the mtgenome, pyrosequencing, quantitative mapping of sequence reads to identify sequence variants and heteroplasmy, as well as *de novo* sequence assembly. These methods have been used to study 40 publicly available HapMap samples of European (CEU) and African (YRI) ancestry to demonstrate a sequencing error rate $< 5.63 \times 10^{-4}$, nucleotide diversity of 1.6×10^{-3} for CEU and 3.7×10^{-3} for YRI, patterns of sequence variation consistent with earlier studies, but a higher rate of heteroplasmy varying between 10% and 50%. These results demonstrate that next-generation sequencing technologies allow interrogation of the mitochondrial genome in greater depth than previously possible which may be of value in biology and medicine.

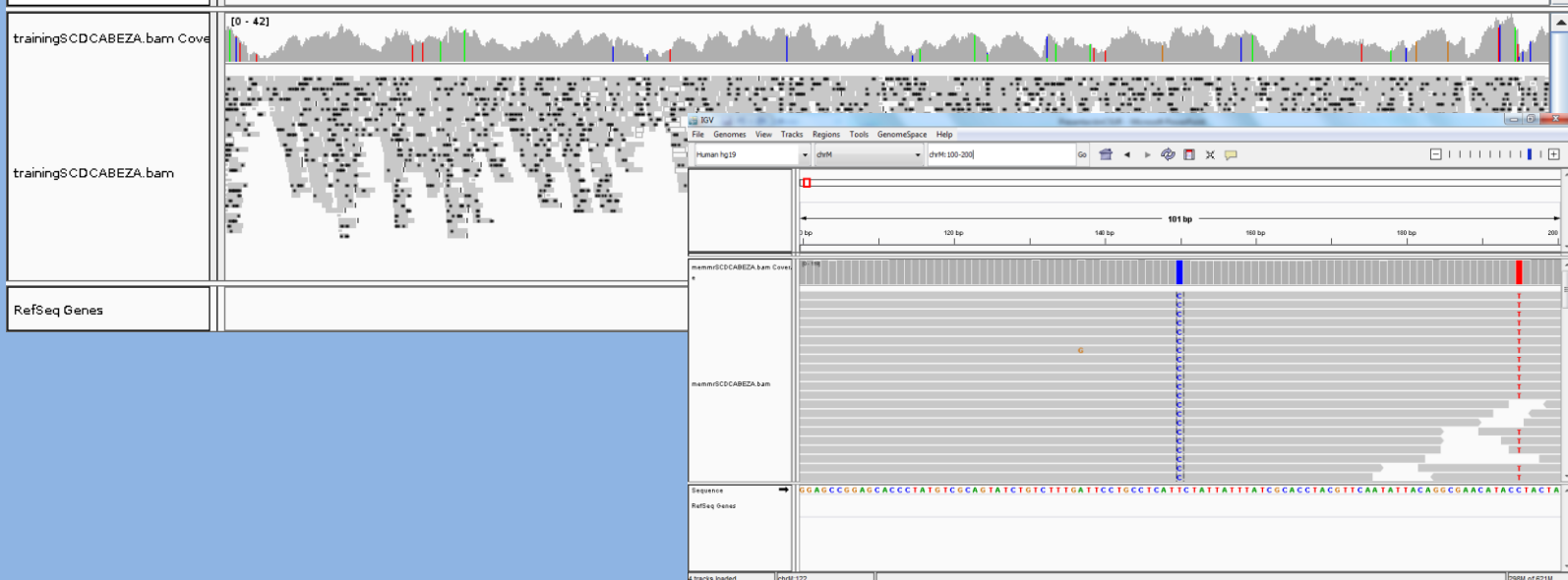
EXOMA



PLAQUETAS



GENOMA





Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

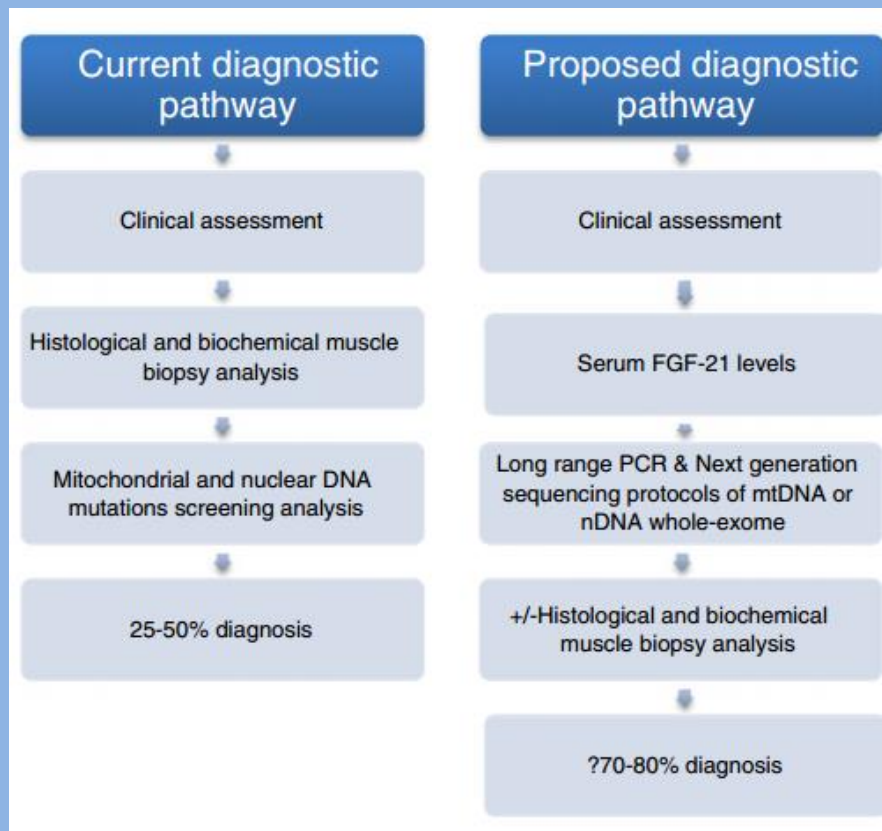


Review

The broadening spectrum of mitochondrial disease: Shifts in the diagnostic paradigm☆☆☆



Christina Liang^a, Kate Ahmad^a, Carolyn M. Sue^{a,b,*}



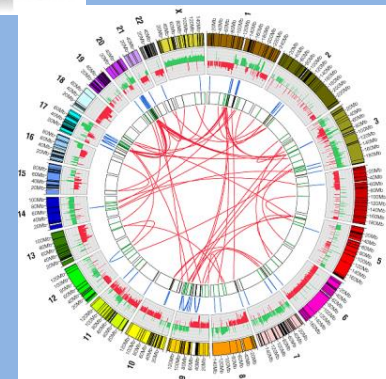
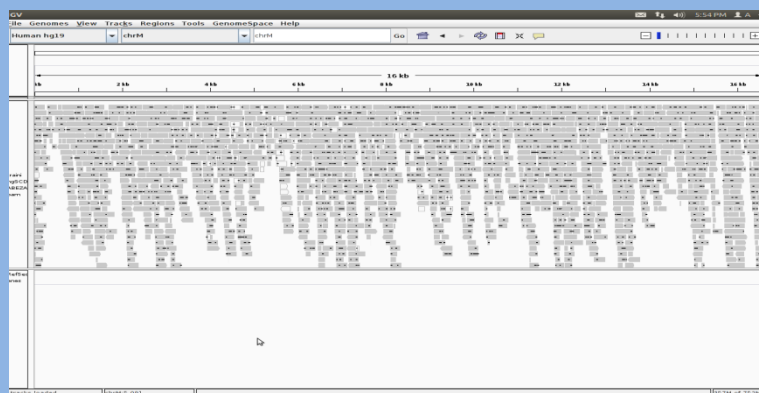
1-4 genes

1-100 genes

Genomas pequeños, ej.
Mitocondria

Exoma
~ 180.000 exones

Genoma humano



Claro gen candidato
Examen de mutaciones
conocidas

Síndrome clínicos:
miopatías, ataxias,
CMT,..

Enfermedades
mitocondriales

Ausencia de hipótesis,
Genes grandes
Paneles de genes

Grandes
reordenamientos
Trastornos
intrónicos



NGS

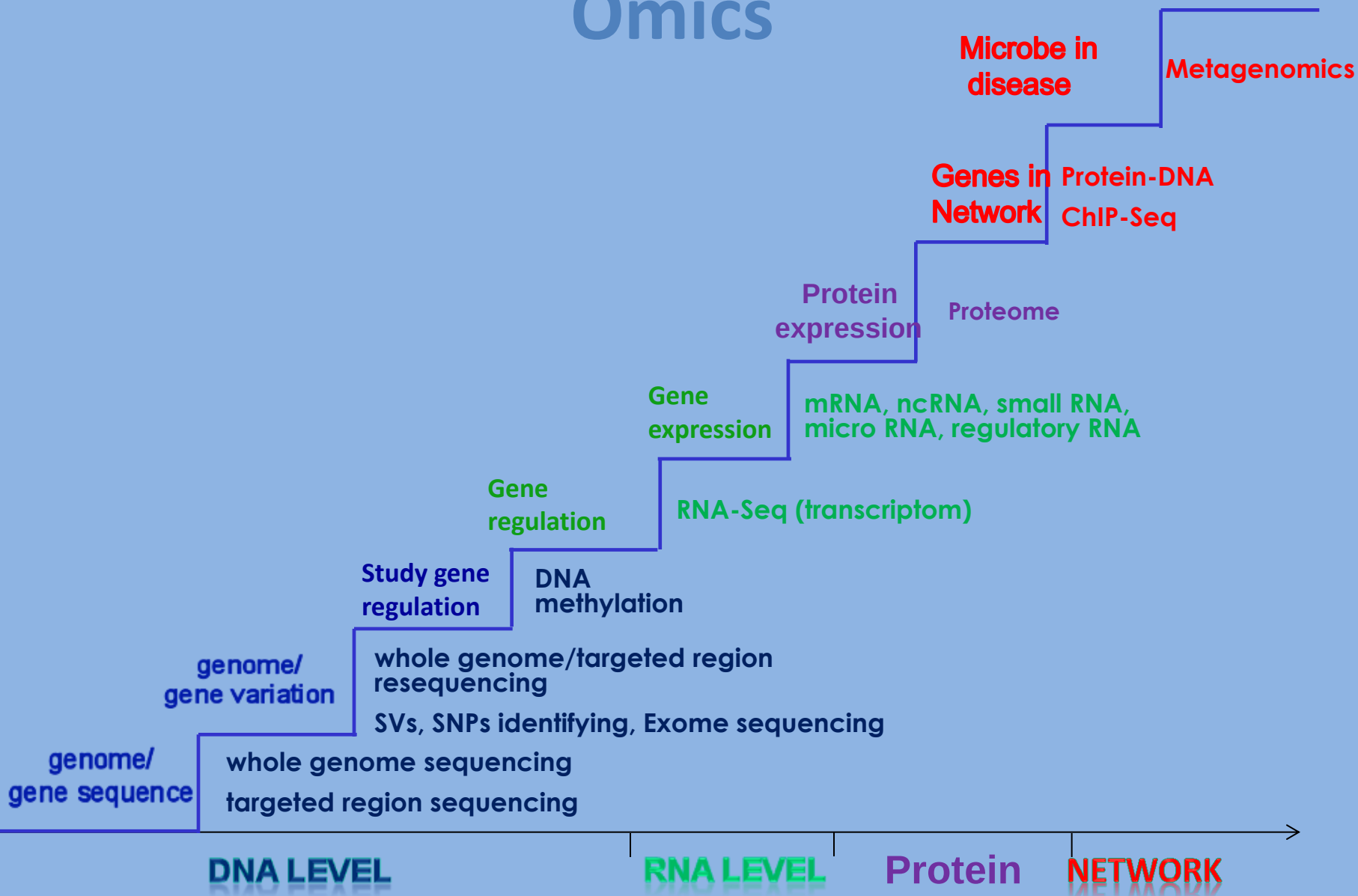
Claro gen candidato: distrofina, DM1
Repeticiones: DM1, DM2
Reordenamientos genómicos: FSHD
Delecciones/inserciones intrónicas

Ausencia de hipótesis
Número alto de genes para un mismo fenotipo
Gen de gran tamaño
Mutaciones puntuales

NGS, BIOPSIA, EMG, ..

Omics

SEQUENCING & SEQUENCING
SEQUENCING & SEQUENCING
SEQUENCING & SEQUENCING
SEQUENCING & SEQUENCING



What can exome sequencing do for you?

Jacek Majewski,^{1,2} Jeremy Schwartzentruber,¹ Emilie Lalonde,^{1,2}
Alexandre Montpetit,¹ Nada Jabado^{2,3}

ABSTRACT

Recent advances in next-generation sequencing technologies have brought a paradigm shift in how medical researchers investigate both rare and common human disorders. The ability cost-effectively to generate genome-wide sequencing data with deep coverage in a short time frame is replacing approaches that focus on specific regions for gene discovery and clinical testing.

an estimated cost of \$2.7 billion.⁶⁻⁸ In 2008, by comparison, a human genome was sequenced over a 5-month period for approximately \$1.5 million⁹ and now, in 2011, sequencing a whole genome is done over a period of a few days and is soon projected to cost less than \$10000. The latter accomplishment was made possible by the commercial launch of the first massively parallel pyro-

sequencing, non-coding RNAs, highly conserved regions of the genome, disease-associated ID blocks or other regions of interest. Because the exome represents only approximately 1% of the genome or about 30 Mb, vastly higher sequence coverage can be readily achieved using second-generation sequencing platforms with considerably less raw sequence and cost than WGS. For example, whereas 90 Gb of sequence is required to obtain 30-fold average coverage of the genome, 75-fold average coverage is achieved for the exome with only 8 Gb of sequence using the current state-of-the-art platforms for targeting.¹⁷⁻¹⁸ However, there are inefficiencies in the targeting process. For example, uneven capture efficiency across exons can result in exons with low sequence coverage, and off-target hybridization means that at least 20% of reads come from genomic DNA outside the exome. In addition, exome capture is not complete. Indeed, the

simply reflect the wish to know what genetic information they are born with. Until 2008 such questions usually remained unanswered because, even for some Mendelian disorders, we did not know how to identify causative mutations within our genome. Indeed, our individual genomes contain variants which may protect us against or increase our susceptibility to our environment and the multiple stressors we encounter during our lifespan. Knowing these variants can perhaps allow us to better prepare or to avoid the negative impacts they might have on our health, lifespan and offspring. In the short years since the first commercial platform became available, NGS has dramatically accelerated multiple areas of genomics research, enabling experiments that previously were not technically feasible or affordable. In this paper we describe the major ongoing applications of NGS as they pertain to WES.

➤ Herramienta diagnóstica que es:

- no invasiva
- económica
- tiempo de realización corto

→ 1er nivel de screening de pacientes con miopatías

➤ Aplicabilidad en casos "no genéticos"

➤ Medicina personalizada

- Un escalón más en el diagnóstico
- Interacción de genes/hallazgos incidentales/farmacogenómica

→ Programas de pacientes sin diagnóstico

In 1977 two landmark articles describing methods for DNA sequencing were published.^{4,5} The approach reported by Sanger and colleagues was further refined and commercialised leading to its dissemination throughout the research community and, ultimately into clinical diagnostics. In an industrial high-throughput configuration, Sanger technology was then used in the sequencing of the first human genome which was completed in 2001 through the Human Genome Project, a 13-year effort with

significant sequencing applications were the genome advantage of increased sequence coverage of regions of interest—such as coding exons of genes—at lower cost and higher throughput compared with random shotgun sequencing methods. Most large-scale methods for targeted sequencing use a variation of a hybrid selection approach. Complementary nucleic acid "baits" are used to "fish" for regions of interest in the total pool of nucleic acids, which can be DNA¹³⁻¹⁵ or RNA.¹⁶ Any subset of the genome can be targeted, including

Probe size	Agilent 120 bases	Roche/Gen 55–105 bases	illumina 95 bases
Target region size	50 Mb	44.1 Mb	62 Mb
Probe type	RNA	DNA	DNA
No. of probes	561824	2.1M	340427
No. of targeted reads	188260	~300 000	201121
Reads on target (%)	> 95	> 95	> 95
On target >200 bases (%)	77	83	80
Reads with > 0.2× mean read coverage (%)	> 95	> 95	> 95

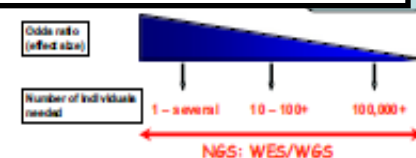


Figure 1 Roadmap for the application of next generation sequencing technologies for the identification of disease-relevant genomic variations.

