Genetics-multistep tumorigenesis genomic integrity & cancer

Sections 11.1-11.8 from Weinberg's 'the biology of Cancer'

Cancer genetics and genomics Selected publications (more of a journal club format)

The Diploid Genome Sequence of an Individual Human

Samuel Levy[1*], Granger Sutton[1], Pauline C. Ng[1], Lars Feuk[2], Aaron L. Halpern[1], Brian P. Walenz[1], Nelson Axelrod[1], Jiaqi Huang[1], Ewen F. Kirkness[1], Gennady Denisov[1], Yuan Lin[1], Jeffrey R. MacDonald[2], Andy Wing Chun Pang[2], Mary Shago[2], Timothy B. Stockwell[1], Alexia Tsiamouri[1], Vineet Bafna[3], Vikas Bansal[3], Saul A. Kravitz[1], Dana A. Busam[1], Karen Y. Beeson[1], Tina C. McIntosh[1], Karin A. Remington[1], Josep F. Abril[4], John Gill[1], Jon Borman[1], Yu-Hui Rogers[1], Marvin E. Frazier[1], Stephen W. Scherer[2], Robert L. Strausberg[1], J. Craig Venter[1]

1 J. Craig Venter Institute, Rockville, Maryland, United States of America, **2** Program in Genetics and Genomic Biology, The Hospital for Sick Children, and Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada, **3** Department of Computer Science and Engineering, University of California San Diego, La Jolla, California, United States of America, **4** Genetics Department, Facultat de Biologia, Universitat de Barcelona, Barcelona, Catalonia, Spain

Presented here is a genome sequence of an individual human. It was produced from ~32 million random DNA fragments, sequenced by Sanger dideoxy technology and assembled into 4,528 scaffolds, comprising 2,810 million bases (Mb) of contiguous sequence with approximately 7.5-fold coverage for any given region. We developed a modified version of the Celera assembler to facilitate the identification and comparison of alternate alleles within this individual diploid genome. Comparison of this genome and the National Center for Biotechnology Information human reference assembly revealed more than 4.1 million DNA variants, encompassing 12.3 Mb. These variants (of which 1,288,319 were novel) included 3,213,401 single nucleotide polymorphisms (SNPs), 53,823 block substitutions (2–206 bp), 292,102 heterozygous insertion/deletion events (indels)(1–571 bp), 559,473 homozygous indels (1–82,711 bp), 90 inversions, as well as numerous segmental duplications and copy number variation regions. Non-SNP DNA variation accounts for 22% of all events identified in the donor, however they involve 74% of all variant bases. This suggests an important role for non-SNP genetic alterations in defining the diploid genome structure. Moreover, 44% of genes were heterozygous for one or more variants. Using a novel haplotype assembly strategy, we were able to span 1.5 Gb of genome sequence in segments >200 kb, providing further precision to the diploid nature of the genome. These data depict a definitive molecular portrait of a diploid human genome that provides a starting point for future genome comparisons and enables an era of individualized genomic information.

Starting to get a view of genome variation & complexity; creates challenges for interpreting cancer genomes

Levy *et al* (2007) *PLoS Biology* **5**:e254

Table 2 | **Sequencing statistics on personal genome projects**

Personal Genome	Platform	Genomic template libraries	No. of reads (millions)	Read length (bases)	Base coverage (fold)	Assembly	Genome coverage (%)*	SNVs in millions (alignment tool)	No. of runs	Estimated cost (US$)
J. Craig Venter	Automated Sanger	MP from BACs, fosmids & plasmids	31.9	800	7.5	*De novo*	N/A	3.21	>340,000	70,000,000
James D. Watson	Roche/454	Frag: 500 bp	93.2[‡]	250[§]	7.4	Aligned*	95[‖]	3.32 (BLAT)	234	1,000,000[¶]
Yoruban male (NA18507)	Illumina/ Solexa	93% MP: 200 bp	3,410[‡]	35	40.6	Aligned*	99.9	3.83 (MAQ)	40	250,000[¶]
		7% MP: 1.8 kb	271	35				4.14 (ELAND)		
Han Chinese male	Illumina/ Solexa	66% Frag: 150–250 bp	1,921[‡]	35	36	Aligned*	99.9	3.07 (SOAP)	35	500,000[¶]
		34% MP: 135 bp & 440 bp	1,029	35						
Korean male (AK1)	Illumina/ Solexa	21% Frag: 130 bp & 440 bp	393[‡]	36	27.8	Aligned*	99.8	3.45 (GSNAP)	30	200,000[¶]
		79% MP: 130 bp, 390 bp & 2.7 kb	1,156	36, 88, 106						
Korean male (SJK)	Illumina/ Solexa	MP: 100 bp, 200 bp & 300 bp	1,647[‡]	35, 74	29.0	Aligned*	99.9	3.44 (MAQ)	15	250,000[¶,#]
Yoruban male (NA18507)	Life/APG	9% Frag: 100–500 bp	211[‡]	50	17.9	Aligned*	98.6	3.87 (Corona-lite)	9.5	60,000[¶,**]
		91% MP: 600–3,500 bp	2,075[‡]	25, 50						
Stephen R. Quake	Helicos BioSciences	Frag: 100–500 bp	2,725[‡]	32[§]	28	Aligned*	90	2.81 (IndexDP)	4	48,000[¶]
AML female	Illumina/ Solexa	Frag: 150–200 bp[‡‡]	2,730[‡,‡‡]	32	32.7	Aligned*	91	3.81[‡‡] (MAQ)	98	1,600,000[III]
		Frag: 150–200 bp[§§]	1,081[‡,§§]	35	13.9		83	2.92[§§] (MAQ)	34	
AML male	Illumina/ Solexa	MP: 200–250 bp[‡‡]	1,620[‡,‡‡]	35	23.3	Aligned*	98.5	3.46[‡‡] (MAQ)	16.5	500,000[III]
		MP: 200–250 bp[§§]	1,351[‡,§§]	50	21.3		97.4	3.45[§§] (MAQ)	13.1	
James R. Lupski CMT male	Life/APG	16% Frag: 100–500 bp	238[‡]	35	29.6	Aligned*	99.8	3.42 (Corona-lite)	3	75,000[¶,¶¶]
		84% MP: 600–3,500 bp	1,211[‡]	25, 50						

*A minimum of one read aligning to the National Center for Biotechnology Information build 36 reference genome. [‡]Mappable reads for aligned assemblies. [§]Average read-length. [‖]D. Wheeler, personal communication. [¶]Reagent cost only. [#]S.-M. Ahn, personal communication. **K. McKernan, personal communication. [‡‡]Tumour sample. [§§]Normal sample. [III]Tumour & normal samples: reagent, instrument, labour, bioinformatics and data storage cost, E. Mardis, personal communication. [¶¶]R. Gibbs, personal communication. AML, acute myeloid leukaemia; BAC, bacterial artificial chromosome; CMT, Charcot–Marie–Tooth disease; Frag, fragment; MP, mate-pair; N/A, not available; SNV, single-nucleotide variant.

Metzger (2010) *Nature Reviews Genetics* **11**:31

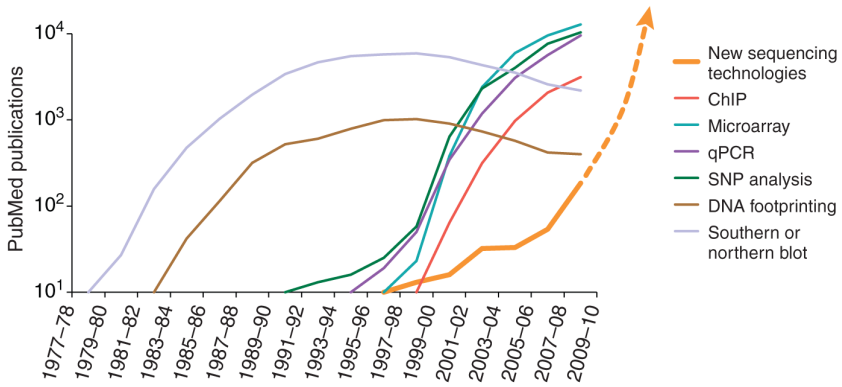
Table 1 | **Comparison of next-generation sequencing platforms**

Platform	Library/ template preparation	NGS chemistry	Read length (bases)	Run time (days)	Gb per run	Machine cost (US$)	Pros	Cons	Biological applications	Refs
Roche/454's GS FLX Titanium	Frag, MP/ emPCR	PS	330*	0.35	0.45	500,000	Longer reads improve mapping in repetitive regions; fast run times	High reagent cost; high error rates in homo-polymer repeats	Bacterial and insect genome *de novo* assemblies; medium scale (<3 Mb) exome capture; 16S in metagenomics	D. Muzny, pers. comm.
Illumina/ Solexa's GA_{II}	Frag, MP/ solid-phase	RTs	75 or 100	4[‡], 9[§]	18[‡], 35[§]	540,000	Currently the most widely used platform in the field	Low multiplexing capability of samples	Variant discovery by whole-genome resequencing or whole-exome capture; gene discovery in metagenomics	D. Muzny, pers. comm.
Life/APG's SOLiD 3	Frag, MP/ emPCR	Cleavable probe SBL	50	7[‡], 14[§]	30[‡], 50[§]	595,000	Two-base encoding provides inherent error correction	Long run times	Variant discovery by whole-genome resequencing or whole-exome capture; gene discovery in metagenomics	D. Muzny, pers. comm.
Polonator G.007	MP only/ emPCR	Non-cleavable probe SBL	26	5[§]	12[§]	170,000	Least expensive platform; open source to adapt alternative NGS chemistries	Users are required to maintain and quality control reagents; shortest NGS read lengths	Bacterial genome resequencing for variant discovery	J. Edwards, pers. comm.
Helicos BioSciences HeliScope	Frag, MP/ single molecule	RTs	32*	8[‡]	37[‡]	999,000	Non-bias representation of templates for genome and seq-based applications	High error rates compared with other reversible terminator chemistries	Seq-based methods	91
Pacific Biosciences (target release: 2010)	Frag only/ single molecule	Real-time	964*	N/A	N/A	N/A	Has the greatest potential for reads exceeding 1 kb	Highest error rates compared with other NGS chemistries	Full-length transcriptome sequencing; complements other resequencing efforts in discovering large structural variants and haplotype blocks	S. Turner, pers. comm.

*Average read-lengths. [‡]Fragment run. [§]Mate-pair run. Frag, fragment; GA, Genome Analyzer; GS, Genome Sequencer; MP, mate-pair; N/A, not available; NGS, next-generation sequencing; PS, pyrosequencing; RT, reversible terminator; SBL, sequencing by ligation; SOLiD, support oligonucleotide ligation detection.

Metzger (2010) *Nature Reviews Genetics* **11**:31

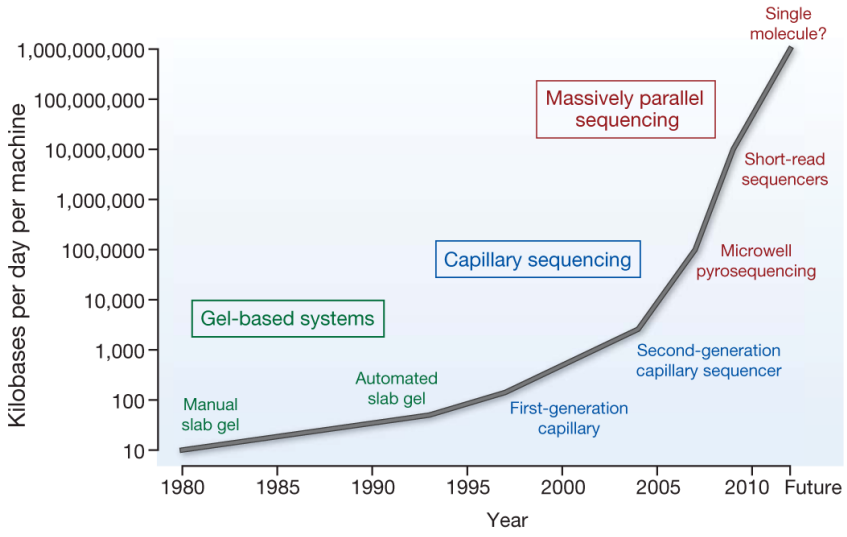
‘Evolution’ of genomic technologies



In general, array-based methods do not provide information on novel somatic mutations (there are exceptions: CGH array, re-sequencing arrays)

Kahvejian *et al* (2007) *Nature Biotechnology* **26**:1125

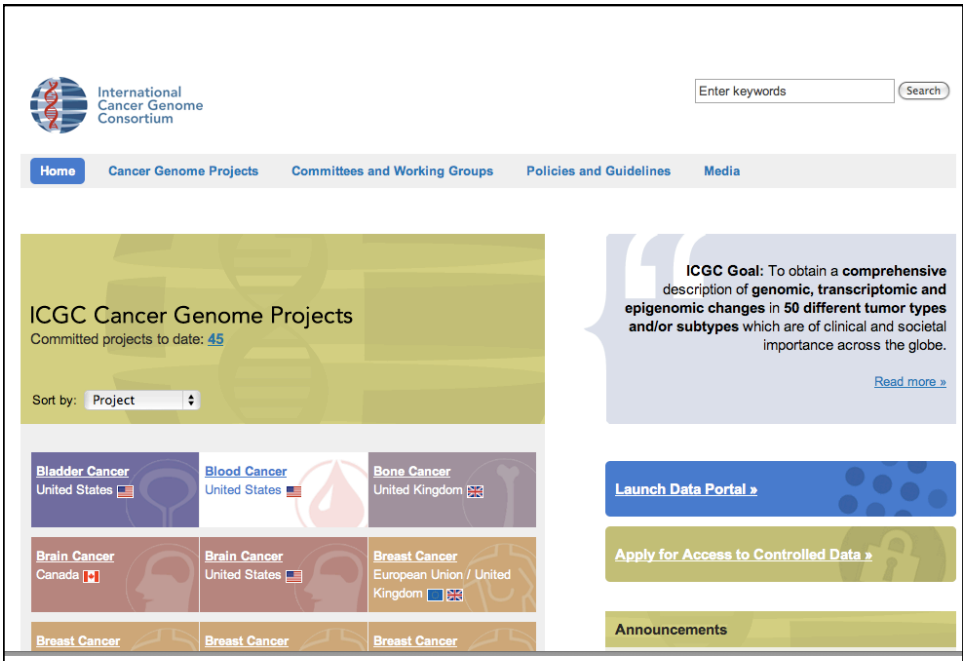
‘Evolution’ of genomic capacity



Kahvejian *et al* (2007) *Nature Biotechnology* **26**:1125

Enter the cancer genome; nextgen platforms provide an unprecedented opportunity to understand cancer genetics and evolution

What are the goals?



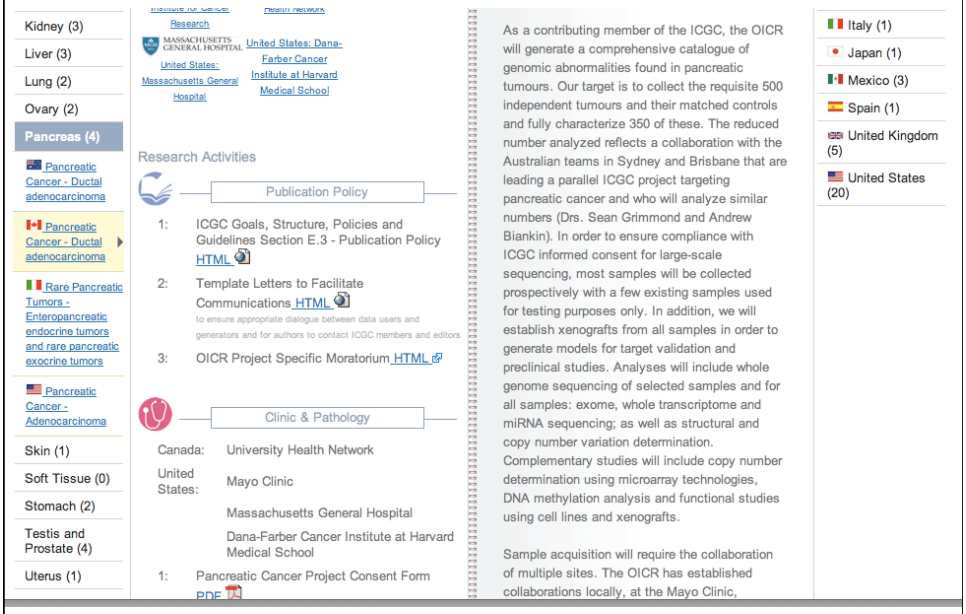
Updates

Currently, the ICGC has received commitments from funding organizations in Asia, Australia, Europe and North America for 39 project teams in 13 jurisdictions to study over 18,000 tumor genomes. Projects that are currently funded are examining tumors affecting the bladder, blood, bone, brain, breast, cervix, colon, head and neck, kidney, liver, lung, oral cavity, ovary, pancreas, prostate, rectum, skin, soft tissues, stomach and uterus. Over time, additional nations and organizations are anticipated to join the ICGC. The genomic analyses of tumors conducted by ICGC members in Australia and Canada (pancreatic cancer), Japan (liver cancer), Spain (blood cancer), the UK (breast, lung and skin cancer) and the USA (blood, brain, breast, colon, kidney, lung, ovarian, rectal, stomach and uterine cancer) are now available through the Data Coordination Center housed on the ICGC website at www.icgc.org.

International network of cancer genome projects. Nature 464, 993-998 (15 April 2010)

Read the article

www.icgc.org



www.icgc.org

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ARTICLES

A comprehensive catalogue of somatic mutations from a human cancer genome

Erin D. Pleasance[1]*, R. Keira Cheetham[2]*, Philip J. Stephens[1], David J. McBride[1], Sean J. Humphray[2], Chris D. Greenman[1], Ignacio Varela[1], Meng-Lay Lin[1], Gonzalo R. Ordóñez[1], Graham R. Bignell[1], Kai Ye[3], Julie Alipaz[4], Markus J. Bauer[2], David Beare[1], Adam Butler[1], Richard J. Carter[2], Lina Chen[1], Anthony J. Cox[2], Sarah Edkins[1], Paula I. Kokko-Gonzales[2], Niall A. Gormley[2], Russell J. Grocock[2], Christian D. Haudenschild[5], Matthew M. Hims[2], Terena James[2], Mingming Jia[1], Zoya Kingsbury[2], Catherine Leroy[1], John Marshall[1], Andrew Menzies[1], Laura J. Mudie[1], Zemin Ning[1], Tom Royce[4], Ole B. Schulz-Trieglaff[2], Anastassia Spiridou[2], Lucy A. Stebbings[1], Lukasz Szajkowski[2], Jon Teague[1], David Williamson[5], Lynda Chin[6], Mark T. Ross[2], Peter J. Campbell[1], David R. Bentley[2], P. Andrew Futreal[1] & Michael R. Stratton[1,7]

Whole genome and transcriptome sequencing of MM metastasis and lymphoblastoid cell lines from same patient

Of 292 somatic base substitutions in coding regions, 187 cause amino acid changes

Pleasance *et al* (2010) *Nature* **463**:191

nature Vol 463|14 January 2010|doi:10.1038/nature08629

ARTICLES

A small-cell lung cancer genome with complex signatures of tobacco exposure

Erin D. Pleasance[1], Philip J. Stephens[1], Sarah O'Meara[1,2], David J. McBride[1], Alison Meynert[3], David Jones[1], Meng-Lay Lin[1], David Beare[1], King Wai Lau[1], Chris Greenman[1], Ignacio Varela[1], Serena Nik-Zainal[1], Helen R. Davies[1], Gonzalo R. Ordoñez[1], Laura J. Mudie[1], Calli Latimer[1], Sarah Edkins[1], Lucy Stebbings[1], Lina Chen[1], Mingming Jia[1], Catherine Leroy[1], John Marshall[1], Andrew Menzies[1], Adam Butler[1], Jon W. Teague[1], Jonathon Mangion[2], Yongming A. Sun[4], Stephen F. McLaughlin[5], Heather E. Peckham[5], Eric F. Tsung[5], Gina L. Costa[5], Clarence C. Lee[5], John D. Minna[6], Adi Gazdar[6], Ewan Birney[3], Michael D. Rhodes[4], Kevin J. McKernan[5], Michael R. Stratton[1,7], P. Andrew Futreal[1] & Peter J. Campbell[1,8]

Whole genome and transcriptome sequencing of SCLC and lymphoblastoid cell lines from same patient

Of 134 somatic base substitutions in coding regions, 98 cause amino acid changes

Pleasance *et al* (2010) *Nature* **463**:184

Staggering range of genomic alterations

Table 1 | Somatic mutations identified in COLO-829

Type of change	Count	Percentage
Substitutions	33,345	100.0
Coding	292	0.9
Silent	105	0.3
Missense	172	0.5
Truncating	15	<0.1
Non-coding	319	1.0
UTR	205	0.6
ncRNA	113	0.3
miRNA	1	<0.1
Intronic	9,543	28.6
Splice	7	<0.1
Other intronic	9,536	28.6
Intergenic	23,191	69.6
Small insertions and deletions	66	100.0
Coding	0	0.0
UTR	2	3.0
Intronic	27	40.9
Intergenic	37	56.1
Rearrangements	37	100.0
Breakpoints	74	
Coding	1	1.4
UTR	0	0.0
Intronic	36	48.6
Intergenic	37	50.0
Classes	37	100.0
Intrachromosomal	34	91.9
Deletions	25	67.6
Inversions	6	16.2
Duplications	2	5.4
Other	1	2.7
Interchromosomal	3	8.1

miRNA, microRNA; ncRNA, non-coding RNA; UTR, untranslated region.

Table 1 | Somatically acquired genomic variants of all classes in a SCLC genome

Variant	Number
Somatic substitution	22,910
Coding	134 (0.6%)
Nonsense	4
Non-synonymous	94
Synonymous	36
Non-coding, transcribed	182 (0.8%)
Untranslated region	119
Non-coding RNA	63
Intronic	6,463 (28%)
Splice site	5
Other intronic	6,458
Intergenic	16,131 (70%)
Insertions and deletions	65
Coding (frameshift)	2 (3%)
Intronic	25 (38%)
Intergenic	38 (58%)
Genomic rearrangements	58
Deletions	18 (31%)
Tandem duplications	9 (16%)
Other non-inverted intrachromosomal rearrangements	9 (16%)
Inverted intrachromosomal rearrangements	15 (26%)
Interchromosomal rearrangements	7 (12%)
Copy number segments	334

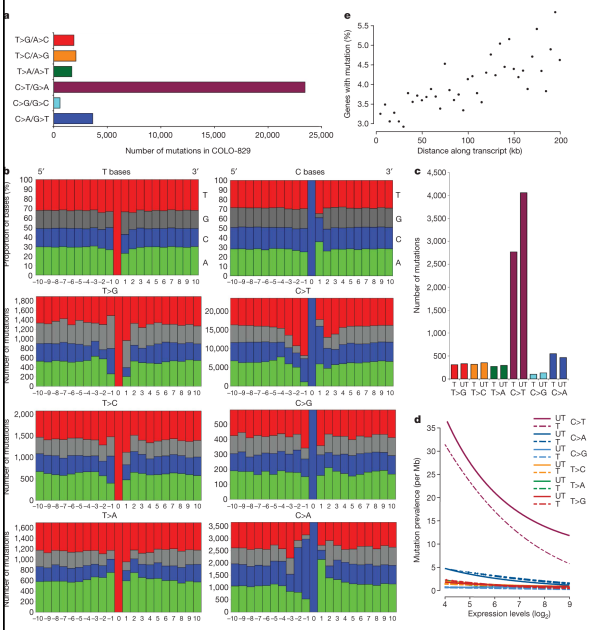
Melanoma

Pleasance *et al* (2010) *Nature* **463**:191

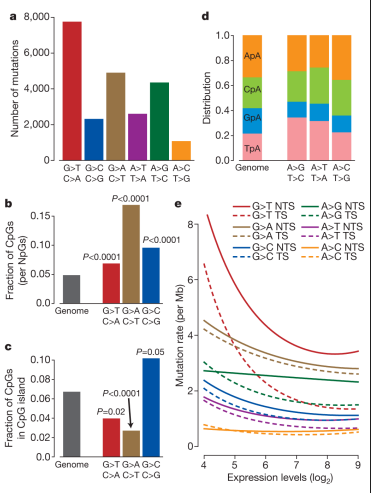
SCLC

Pleasance *et al* (2010) *Nature* **463**:184

Melanoma



SCLC



Different mutational signatures; similar repair signatures

Pleasance *et al* (2010) *Nature* **463**:191

Pleasance *et al* (2010) *Nature* **463**:184

Doing the math

- Lung cancer after 50 pack-years (7,300 cigarettes/year, pack a day)
- Mutation spectra here similar to primary lung cancers
- Clone of cells that gives rise to cancer accumulates 1 mutation per 15 cigarettes
- Substantial mutation over the bronchial tree (cells not cancerous)

Pleasance *et al* (2010) *Nature* **463**:184

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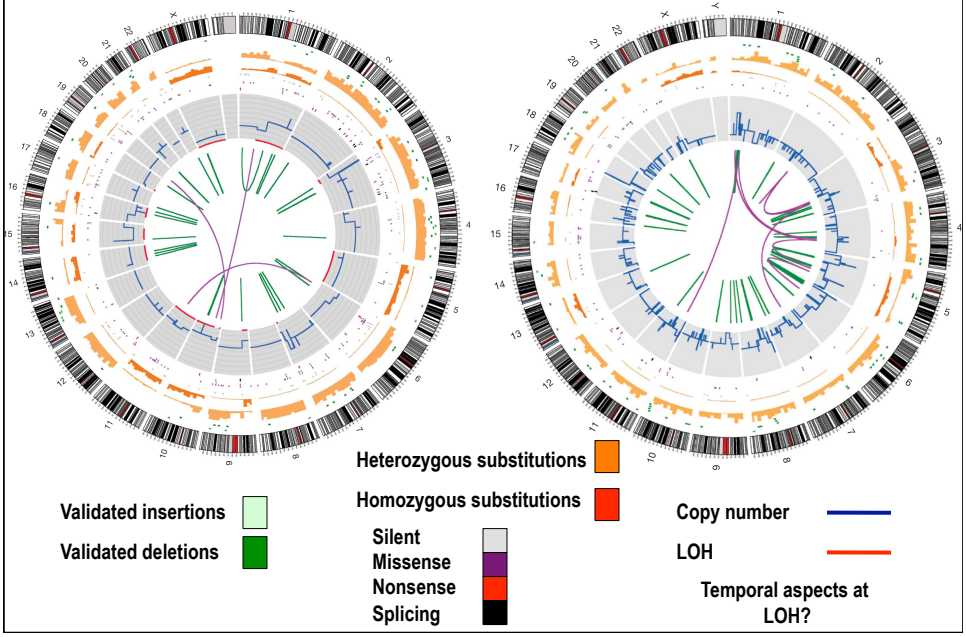


- ❑ Need to distinguish ‘drivers’ from passengers’
- ❑Known mutations or pathways
- ❑Novel pathways or mechanisms; back to the bench

Exome sequencing: higher throughput but limited genome coverage

LETTERS

nature genetics

Exome sequencing identifies GRIN2A as frequently mutated in melanoma

Xiaomu Wei[1], Vijay Walia[1,12], Jimmy C Lin[2,12], Jamie K Teer[3], Todd D Prickett[1], Jared Gartner[1], Sean Davis[4], NISC Comparative Sequencing Program[5], Katherine Stemke-Hale[6], Michael A Davies[6,7], Jeffrey E Gershenwald[8,9], William Robinson[10], Steven Robinson[10], Steven A Rosenberg[11] & Yardena Samuels[1]

Opportunities for gene and pathway discovery

Wei *et al* (2011) *Nature Genetics* **43**:4442

Targeted sequencing of the exome

- ❑ 14 matched normal and metastatic tumor DNAs (untreated individuals); 'discovery set'
- ❑ Targeted exon capture (37Mb/genome; ~1%)
- ❑ Exons and flanking regions from 20,000 genes
- ❑ 180-fold coverage (12Gb/genome)
- ❑ Multiple filtering steps to distinguish driver/ passenger mutations
- ❑ Further validation by targeted re-sequencing in additional melanoma samples

Limiting the genome content analyzed can afford much higher coverage

Wei *et al* (2011) *Nature Genetics* **43**:4442

Genes with frequent mutations in melanoma

Table 2 Whole exome sequencing in melanoma revealed sixteen highly mutated genes

			Exome capture ($n = 14$)			Prevalence screen ($n = 38$)			Combined exome capture and prevalence screen ($n = 52$)		
Gene name	UCSC ID	*P*	No. of non-synonymous mutations	No. of tumors affected	% of tumors affected	No. of non-synonymous mutations	No. of tumors affected	% of tumors affected	No. of non-synonymous mutations	No. of tumors affected	% of tumors affected
BRAF	uc003vwc.2	4.80×10^{-5}	7	7	50.0	27	27	71.1	34	34	65.4
GRIN2A	uc002czq.1	6.36×10^{-3}	6	6	42.9	11	11	28.9	17	17	32.7
CCDC63	uc001trv.1	3.34×10^{-3}	4	4	28.6	2	2	5.3	6	6	11.5
TMEM132B	uc001uhe.1	7.59×10^{-3}	5	4	28.6	5	5	13.2	10	9	17.3
ZNF831	uc002yan.1	1.29×10^{-2}	5	4	28.6	5	5	13.2	10	9	17.3
PLCB4	uc010gbx.1	4.39×10^{-2}	4	4	28.6	4	4	10.5	8	8	15.4
AKR1B10	uc003vrr.1	5.21×10^{-3}	3	3	21.4	1	1	2.6	4	4	7.7
TAS2R60	uc003wdb.1	5.46×10^{-3}	3	3	21.4	2	2	5.3	5	5	9.6
KHDRBS2	uc003peg.2	7.26×10^{-3}	3	3	21.4	2	2	5.3	5	5	9.6
PTPRO	uc001rda.1	9.09×10^{-3}	3	3	21.4	1	1	2.6	4	4	7.7
SYT4	uc002law.1	1.23×10^{-2}	3	3	21.4	1	1	2.6	4	4	7.7
UGT2B10	uc003hee.1	2.13×10^{-2}	3	3	21.4	1	1	2.6	4	4	7.7
SLC6A11	uc003bvz.1	2.84×10^{-2}	3	3	21.4	0	0	0.0	3	3	5.8
SLC17A5	uc003phn.2	7.91×10^{-3}	4	3	21.4	0	0	0.0	4	3	5.8
C12orf63	uc001tet.1	4.46×10^{-2}	4	3	21.4	2	2	5.3	6	5	9.6
PCDHB8	uc003liu.1	4.80×10^{-2}	3	3	21.4	1	1	2.6	4	4	7.7

Based on genome build hg 18 (NCBI 36.1).

Identified 16 genes with >2 distinct mutations; further validation in 38 samples; *GRIN2A* had a very high frequency (1/3)

Wei *et al* (2011) *Nature Genetics* **43**:4442

- Unprecedented ability to understand cancer evolution
- New insight & hypotheses for cancer biology
  - Mutagenesis
  - Repair
  - Pathways
- Therapeutics & treatment
- Personalized therapy

- Need to consider germline variation as well
- GWAS studies and the role of rare alleles; the few vs. the many

GWAS: discovery of rare alleles

Genome-wide association study identifies a new melanoma susceptibility locus at 1q21.3

Stuart MacGregor[1], Grant W Montgomery[1], Jimmy Z Liu[1], Zhen Zhen Zhao[1], Anjali K Henders[1], Mitchell Stark[1], Helen Schmid[2], Elizabeth A Holland[2], David L Duffy[1], Mingfeng Zhang[3], Jodie N Painter[1], Dale R Nyholt[1], Judith A Maskiell[4], Jodie Jetann[5], Megan Ferguson[5], Anne E Cust[4,6], Mark A Jenkins[4], David C Whiteman[1], Håkan Olsson[7,33], Susana Puig[8,9], Giovanna Bianchi-Scarrà[10], Johan Hansson[11], Florence Demenais[12,13], Maria Teresa Landi[14], Tadeusz Dębniak[15], Rona Mackie[16], Esther Azizi[17], Brigitte Bressac-de Paillerets[12,18], Alisa M Goldstein[14], Peter A Kanetsky[19,20], Nelleke A Gruis[21], David E Elder[22], Julia A Newton-Bishop[23], D Timothy Bishop[23], Mark M Iles[23], Per Helsing[24], Christopher I Amos[25], Qingyi Wei[25], Li-E Wang[25], Jeffrey E Lee[26], Abrar A Qureshi[3,27], Richard F Kefford[2], Graham G Giles[28], Bruce K Armstrong[6], Joanne F Aitken[5], Jiali Han[3,27,29], John L Hopper[4], Jeffrey M Trent[30,31], Kevin M Brown[32], Nicholas G Martin[1], Graham J Mann[2] & Nicholas K Hayward[1]

We performed a genome-wide association study of melanoma in a discovery cohort of 2,168 Australian individuals with melanoma and 4,387 control individuals. In this discovery phase, we confirm several previously characterized melanoma-associated loci at *MC1R*, *ASIP* and *MTAP–CDKN2A*. We selected variants at nine loci for replication in three independent case-control studies (Europe: 2,804 subjects with melanoma, 7,618 control subjects; United States 1: 1,804 subjects with melanoma, 1,026 control subjects; United States 2: 585 subjects with melanoma, 6,500 control subjects). The combined meta-analysis of all case-control studies identified a new susceptibility locus at 1q21.3 (rs7412746, $P = 9.0 \times 10^{-11}$, OR in combined replication cohorts of 0.89 (95% CI 0.85–0.95)). We also show evidence suggesting that melanoma associates with 1q42.12 (rs3219090, $P = 9.3 \times 10^{-8}$). The associated variants at the 1q21.3 locus span a region with ten genes, and plausible candidate genes for melanoma susceptibility include *ARNT* and *SETDB1*. Variants at the 1q21.3 locus do not seem to be associated with human pigmentation or measures of nevus density.

MacGregor *et al* (2011) *Nature Genetics* **43**:1114

Identification of SNPs

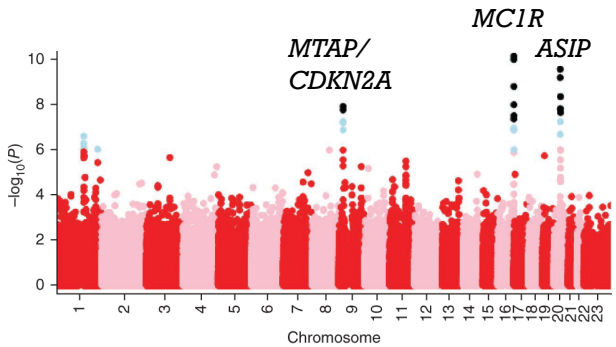
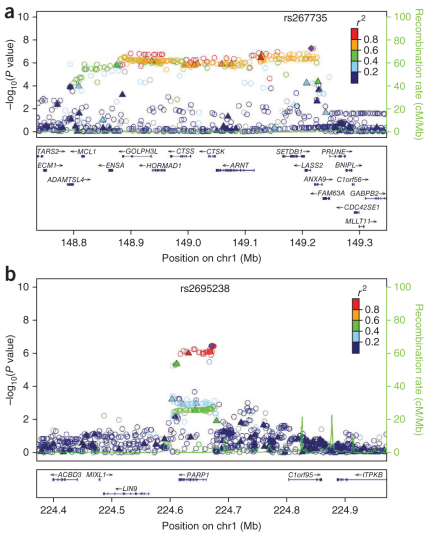


Figure 1 Association results for SNPs directly genotyped in all Australian samples. SNPs with *P* values exceeding genome-wide significance ($P < 5 \times 10^{-8}$) are shown in black, and SNPs with $5 \times 10^{-8} < P < 1 \times 10^{-6}$ are shown in blue. The *y* axis is truncated at 1×10^{-9}; however, some SNPs from previously identified loci exceeded this threshold (specifically at ~88 Mb on chromosome 16 near *MC1R* and at the *ASIP* locus at 33 Mb on chromosome 20. The significant genome-wide signal on chromosome 9 is in the vicinity of the *MTAP/CDKN2A* region.

MacGregor *et al* (2011) *Nature Genetics* **43**:1114

Detailed chromosome 1 SNP analysis



SETDB1 appears as the leading candidate; accounts for only 0.1% of genetic risk

Figure 2 Discovery sample association results at two loci on chromosome 1 for both SNPs directly genotyped in all Australian samples and imputed SNPs. (**a**,**b**) Genotyped SNPs are indicated by filled-in triangles and imputed SNPs by empty circles. The top-ranked SNP at each locus is shown as a filled-in purple diamond. (This SNP is an imputed SNP at both loci.) Imputation *P* values for all SNPs are plotted. Note imputed and genotyped *P* values for genotyped SNPs differ slightly, because for the imputed result, analysis was based on dosage scores, whereas with genotyped SNPs, hard genotype calls were used. Association results shown are for the chromosome 1 locus near 149 Mb (**a**) and SNPs in the vicinity of the *PARP1* association signal (**b**). The color scheme indicates linkage disequilibrium between the most strongly associated SNPs for the 149 Mb and *PARP1* regions (shown in purple, rs267735 and rs2695238, respectively) and other genotyped SNPs in the two regions.

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- ❑Development of resistance
- ❑The next step in clonal evolution

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BIOLOGY OF NEOPLASIA

Dissecting Therapeutic Resistance to RAF Inhibition in Melanoma by Tumor Genomic Profiling

Nikhil Wagle, Caroline Emery, Michael F. Berger, Matthew J. Davis, Allison Sawyer, Panisa Pochanard, Sarah M. Kehoe, Cory M. Johannessen, Laura E. MacConaill, William C. Hahn, Matthew Meyerson, and Levi A. Garraway

A B S T R A C T

A detailed understanding of the mechanisms by which tumors acquire resistance to targeted anticancer agents should speed the development of treatment strategies with lasting clinical efficacy. RAF inhibition in *BRAF*-mutant melanoma exemplifies the promise and challenge of many targeted drugs; although response rates are high, resistance invariably develops. Here, we articulate overarching principles of resistance to kinase inhibitors, as well as a translational approach to characterize resistance in the clinical setting through tumor mutation profiling. As a proof of principle, we performed targeted, massively parallel sequencing of 138 cancer genes in a tumor obtained from a patient with melanoma who developed resistance to PLX4032 after an initial dramatic response. The resulting profile identified an activating mutation at codon 121 in the downstream kinase MEK1 that was absent in the corresponding pretreatment tumor. The $MEK1^{C121S}$ mutation was shown to increase kinase activity and confer robust resistance to both RAF and MEK inhibition in vitro. Thus, $MEK1^{C121S}$ or functionally similar mutations are predicted to confer resistance to combined MEK/RAF inhibition. These results provide an instructive framework for assessing mechanisms of acquired resistance to kinase inhibition and illustrate the use of emerging technologies in a manner that may accelerate personalized cancer medicine.

J Clin Oncol 29:3085-3096.

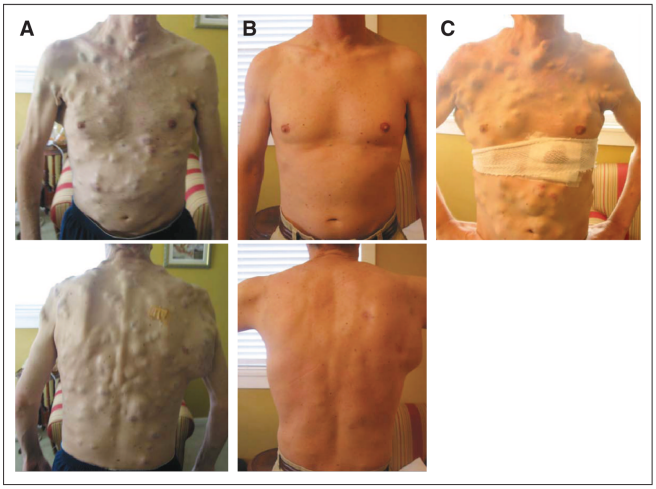

Fig 2. A 38-year-old man with *BRAF*-mutant melanoma and miliary, subcutaneous metastatic deposits. Photographs were taken (A) before initiation of PLX4032, (B) after 15 weeks of therapy with PLX4032, and (C) after relapse, after 23 weeks of therapy.

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Table 1. Somatic Alterations in the PLX4032-Resistant and PLX4032-Sensitive Tumor Samples in a Patient With Metastatic Melanoma

	PLX4032-Resistant Tumor				
Gene	Genomic Change	Protein Change	Mutation Type	Allele Frequency (%)	PLX4032-Sensitive Tumor Protein Change
BRAF	g.chr7:140099605A>T	p.V600E	Missense	37	p.V600E
BRCA1	g.chr17:38497417C>T	p.E1172E	Synonymous	75	p.E1172E
BRCA1	g.chr17:38499682G>A	p.T417T	Synonymous	77	p.T417T
ERBB4	g.chr2:211956862C>T	p.G1217E	Missense	24	p.G1217E
FGFR4	g.chr5:176454998C>T	p.I527I	Synonymous	20	p.I527I
FLT1	g.chr13:27903435C>T	p.A276T	Missense	66	p.A276T
MEK1	g.chr15:64516208G>C	p.C121S	Missense	16	WT
PDGFRB	g.chr5:149477517G>A	p.L998L	Synonymous	57	p.L998L
PTPRD	g.chr9:8490976C>T	p.E623K	Missense	55	p.E623K
PTPRD	g.chr9:8497431G>A	p.P503L	Missense	55	p.P503L
RET	g.chr10:42930184G>C	p.K710N	Missense	28	WT
RUNX1T1	g.chr8:93052172C>T	p.D477N	Missense	76	p.D477N
TERT	g.chr5:1331863C>T	p.E727K	Missense	58	p.E727K
TERT	g.chr5:1331864C>T	p.T726T	Synonymous	58	p.T726T

NOTE. All the exons from the 138 cancer genes were targeted for sequencing by massively parallel sequencing in the PLX4032-resistant sample. Fourteen somatic base substitutions were found. The original (PLX4032-sensitive) sample was queried for the presence of these mutations using mass spectrometric genotyping, demonstrating WT *MEK1* and *RET*.

Abbreviation: WT, wild type.

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Table 2. Exemplary Mechanisms of Acquired Resistance to Kinase Inhibitors

Targeted Agent	Target Gene	Acquired Resistance via Secondary Mutation, Amplification, or Activation of Target	Acquired Resistance via Bypass	Acquired Resistance via Downstream Mutation
Imatinib				
	ABL	T315I Y253F/H E255K/V *ABL* amplification T670I V654A D816A/G/H/V D820A/E/G/Y	*IGF1R* amplification AXL overexpression*†	
	KIT	Y823D *KIT* amplification		
	PDGFRA	T674I		
Gefitinib or erlotinib	*EGFR*	T790M D761Y L747S T854A *EGFR* amplification*	*MET* amplification HGF overexpression*† IGFBP3 loss*†	
Trastuzumab	*HER2*			
Lapatinib	*HER2/EGFR*			
PKC412	*FLT3* *FGFR*	N676K		
AZD6044	*MEK1*	MEK1 P124L *BRAF* amplification*		
PLX4032	*BRAF*	NRAS Q61K	COT overexpression† PDGFRβ overexpression† CRAF overexpression*† AXL overexpression*† HER2 overexpression*†	MEK1 C121S
Crizotinib	*ALK/MET*	L1196M C1156Y F1174L		

Abbreviations: IGF1R, insulin-like growth factor 1 receptor; HGF, hepatocyte growth factor; IGFBP3, insulin-like growth factor receptor binding protein-3; PDGFRβ, platelet-derived growth factor β; HER2, human epidermal growth factor receptor 2.

*Mechanisms that have been described in vitro.

†Nongenetic mechanisms.

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KEY CONCEPTS:

- ❑ Genome complexity
- ❑ Understanding contribution of germline variation
- ❑ Drivers vs. Passengers
- ❑ Haploid coverage and the identification of rare events (clones)
- ❑ Clonal evolution & development of resistance
- ❑ Epigenetic changes (need to analyze in parallel); just becoming possible
- ❑ Emerging therapies dependent on genetic state of the tumor
- ❑ Move towards PERSONALIZED THERAPY

- ❑ Further reading (if you're interested)
- ❑ Implementing genomics into patient treatment

Leading Edge
Review

Cell

The Genetic Basis for Cancer Treatment Decisions

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Personalized cancer medicine is based on increased knowledge of the cancer mutation repertoire and availability of agents that target altered genes or pathways. Given advances in cancer genetics, technology, and therapeutics development, the timing is right to develop a clinical trial and research framework to move future clinical decisions from heuristic to evidence-based decisions. Although the challenges of integrating genomic testing into cancer treatment decision making are wide-ranging and complex, there is a scientific and ethical imperative to realize the benefits of personalized cancer medicine, given the overwhelming burden of cancer and the unprecedented opportunities for advancements in outcomes for patients.

Dancey *et al* (2012) *Cell* **Online first**: February 3