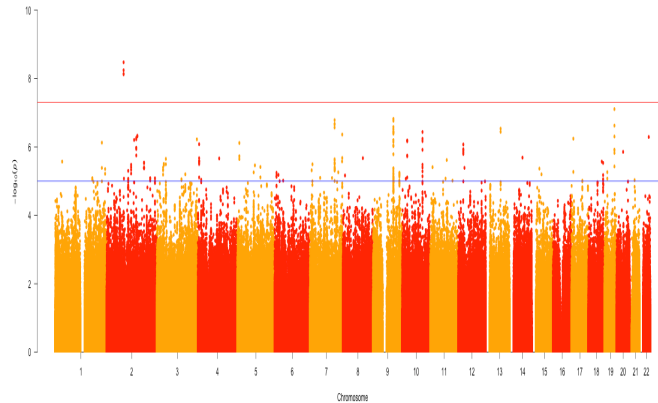
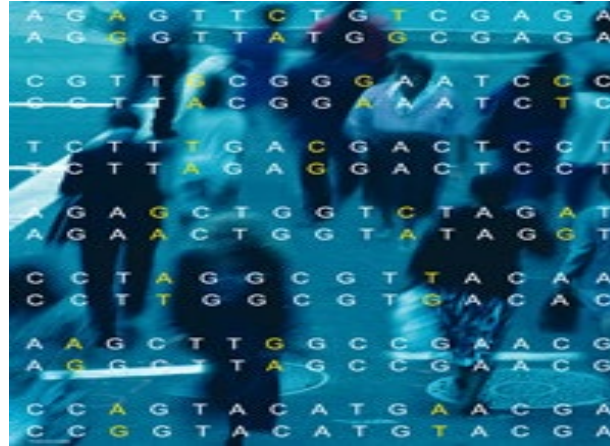


Genomics Analysis for Cleft Lip and Palate



Azeez Butali DDS, PhD, FICD, FAMedS, FAAAS, FMCDs (Honoris Causa)
Presidential Early Career Award for Scientists and Engineers 2025 Awardee
Fulbright Scholar 2025-2026

Gilbert Lilly Endowed Professor of Diagnostic Sciences
Professor of Craniofacial Genetics

Professor, Department of Pediatrics, Carver College of Medicine

Professor, Iowa Institute of Oral Health Research

Professor, Iowa Institute of Human Genetics

Director, African Craniofacial Anomalies Network (AfriCRAN)

College of Dentistry, University of Iowa

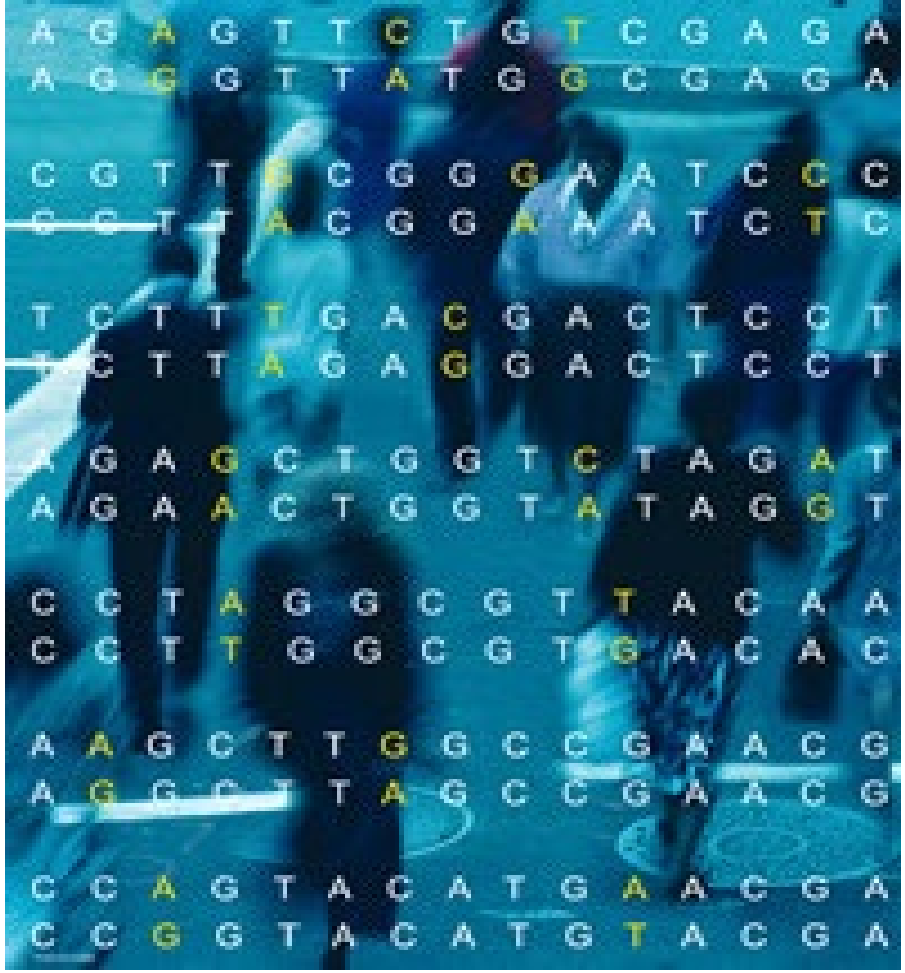
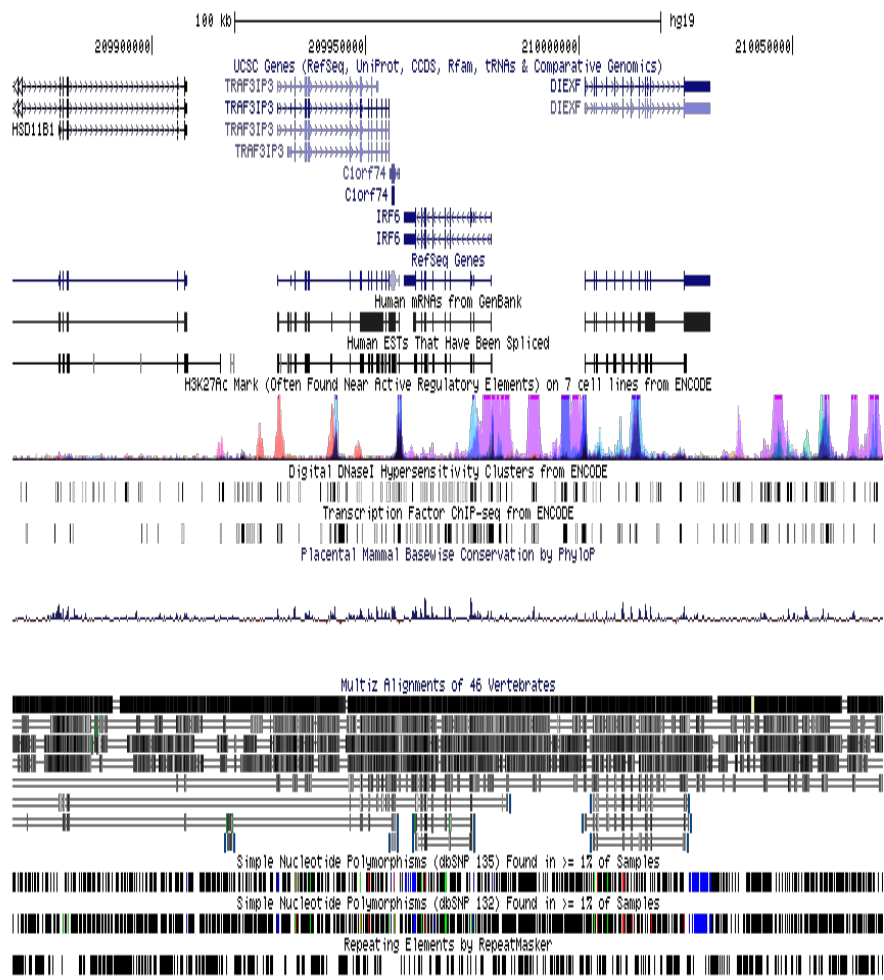


IADR San Diego 2026

25/03/26

Introduction

Genomics is the study entire genome at the same time.



Orofacial cleft (OFC) is one of the most common birth defects and serve as sentinel for other birth defects.

Epidemiology

Clinical and psychological impact

**Cultural beliefs
and impact**

Treatment



Financial Impact

Strategies for genomics analyses

1. Genome-wide association studies
2. Exome sequencing
3. Whole Genome Sequencing
4. Copy Number Variations

Strategies for genomics analyses

- 1. Genome-wide association studies**
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Genome wide association studies

Sequence variation:

Single Nucleotide polymorphisms (SNP)

e.g. GT**A**GCTGTGG to G**T**C**G**CTGTGG

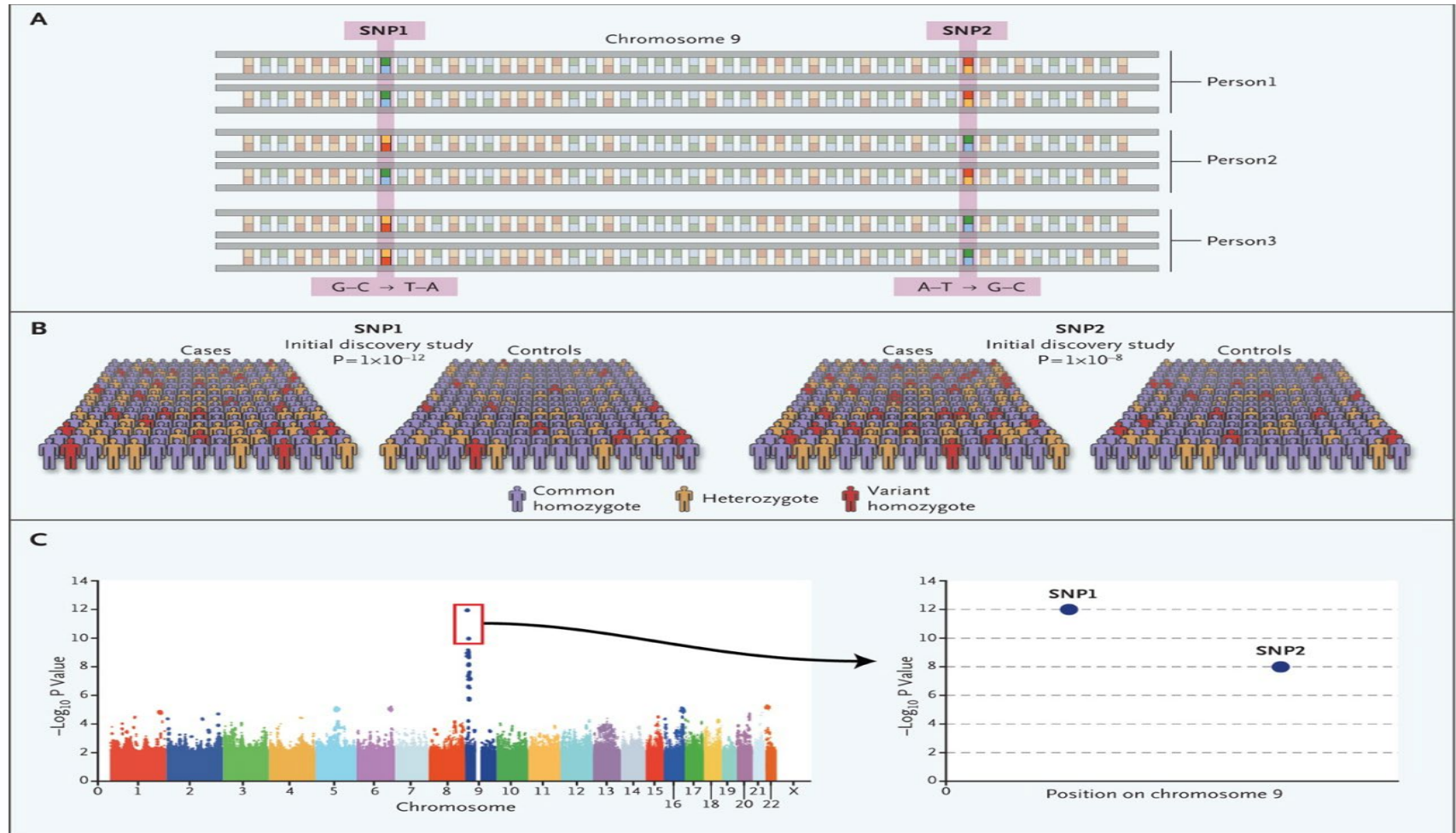
SNP $\geq 1\%$ of the population.

~90% of all human genetic variation.

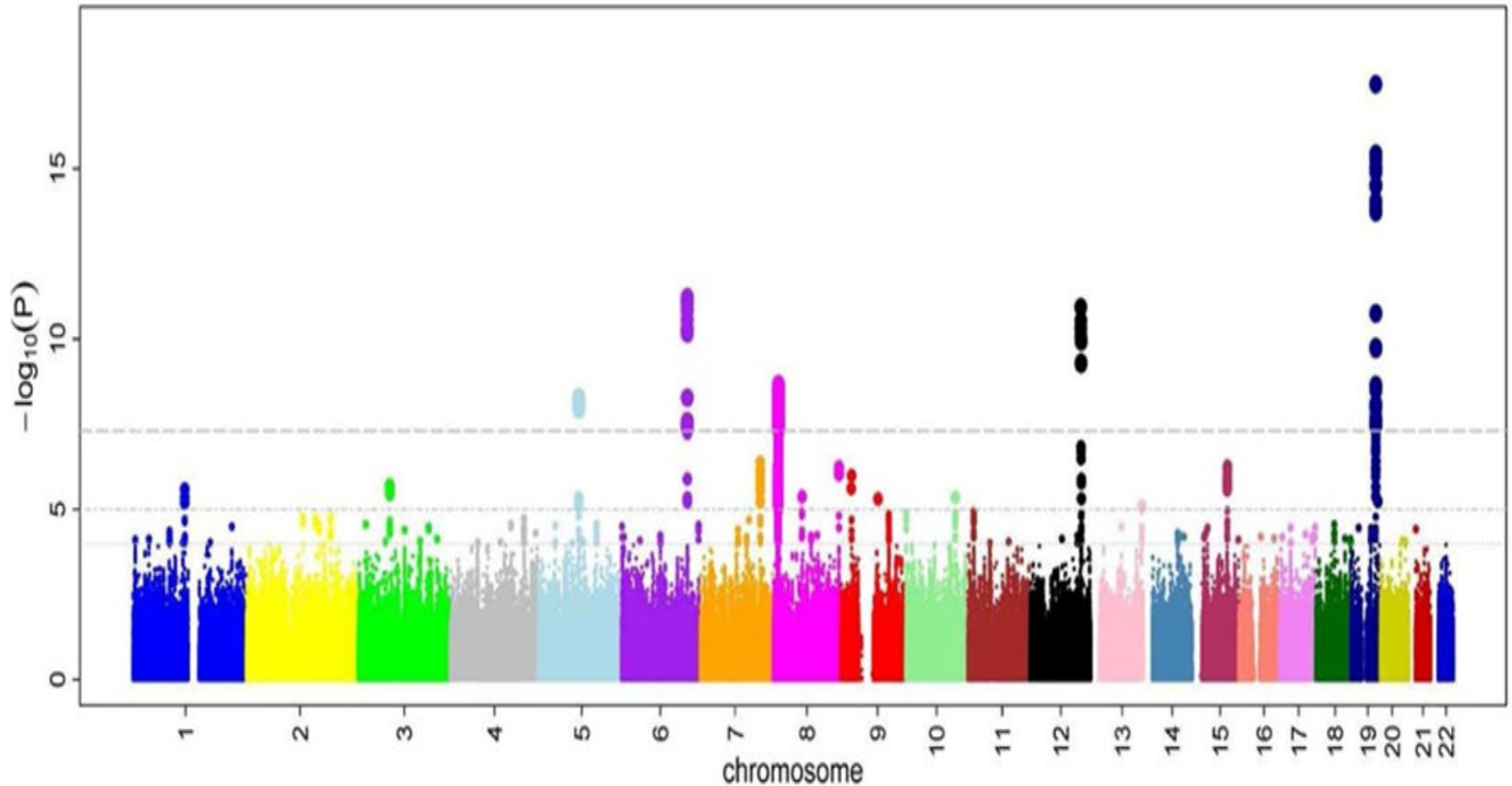
SNPs occur every 100 – 300 bases along the human genome sequence.



Genome wide association studies (GWAS) are conducted to identify SNPs across the genome that are associated with diseases or traits.



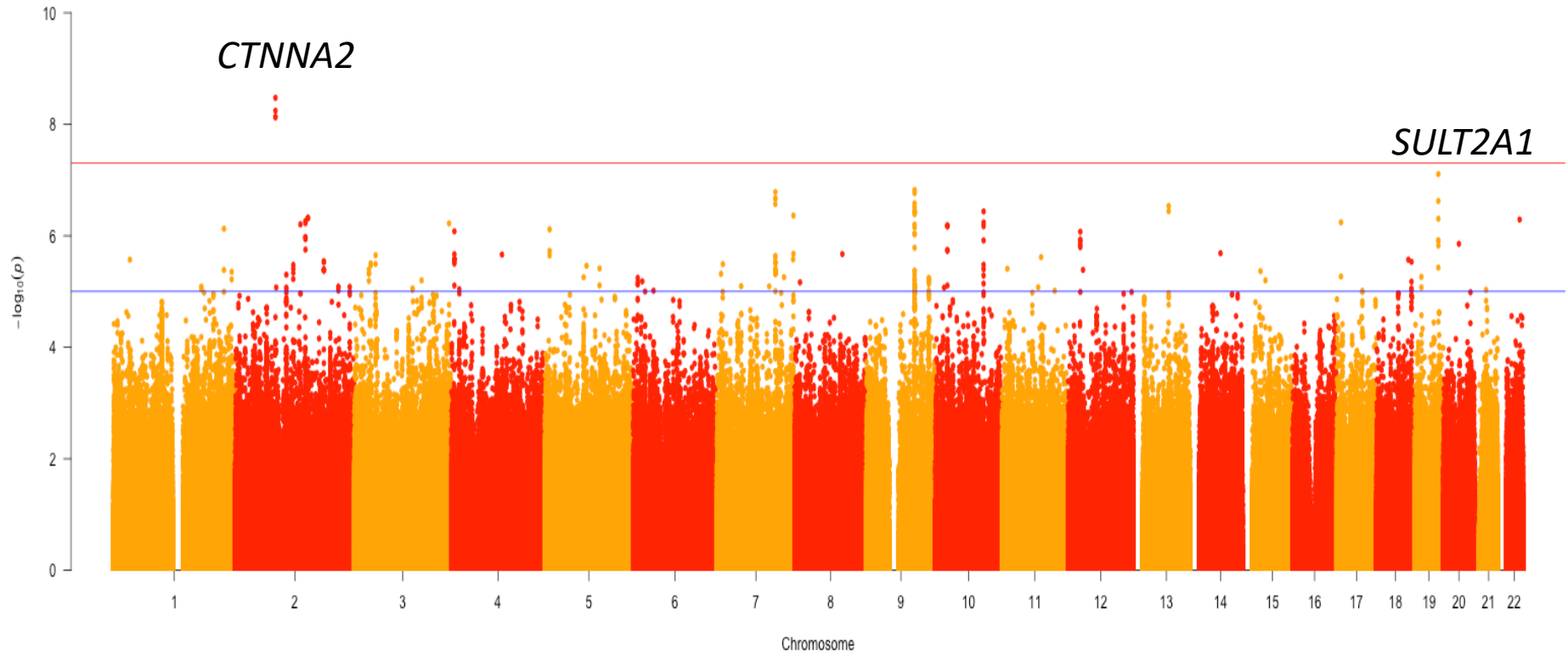
Genome wide association studies (GWAS) are conducted to identify SNPs across the genome that are associated with diseases or traits.



Over 4,500 GWAS publications and 55,000 SNPs that are GWAS significant have been reported to date – Loos, 2020 (Nature Communications)



GWAS of 205 cases of isolated CP and 2159 related and unrelated controls identified 2 new loci and a locus near *PTCH1*.



Mansilla et al., 2011. Contributions of *PTCH* Gene Variants to Isolated Cleft Lip and Palate. Cleft Palate Craniofacial Journal.

Wu et al., 2017 also identified *PTCH1* as GWAS significant in isolated CP. Nature Genetics

Butali et al., 2019. Human Molecular Genetics.

Several GWAS and candidate genes studies have been conducted for CL/P and over 50 loci reported.

Studies	Population
Birnbaum et al.,2009	European
Grant et al., 2009	European
Mangold et.al.,2010	European
Beaty et al.,2010	European
	Asian
Sun et al., 2015	Asian
Leslie et al., 2016a	Multi-ethnic
Leslie et al., 2016b	Multi-ethnic
Mangold et al., 2016	European
Wu et al., 2017	Asians
<i>Butali et al., 2019</i>	<i>Africans</i>

MENU **nature genetics**

Letter | Published: 02 May 2010

A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4

Terri H Beaty✉, Jeffrey C Murray, [...] Alan F Scott

Nature Genetics **42**, 525–529(2010) | Cite this article

288 Accesses | 321 Citations | 6 Altmetric | Metrics

MENU **nature genetics**

Brief Communication | Published: 05 August 2012

Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci

Kerstin U Ludwig, Elisabeth Mangold✉, [...] Markus M Nöthen

Nature Genetics **44**, 968–971(2012) | Cite this article

167 Accesses | 188 Citations | 11 Altmetric | Metrics

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Volume 28, Issue 6
15 March 2019

Genomic analyses in African populations identify novel risk loci for cleft palate

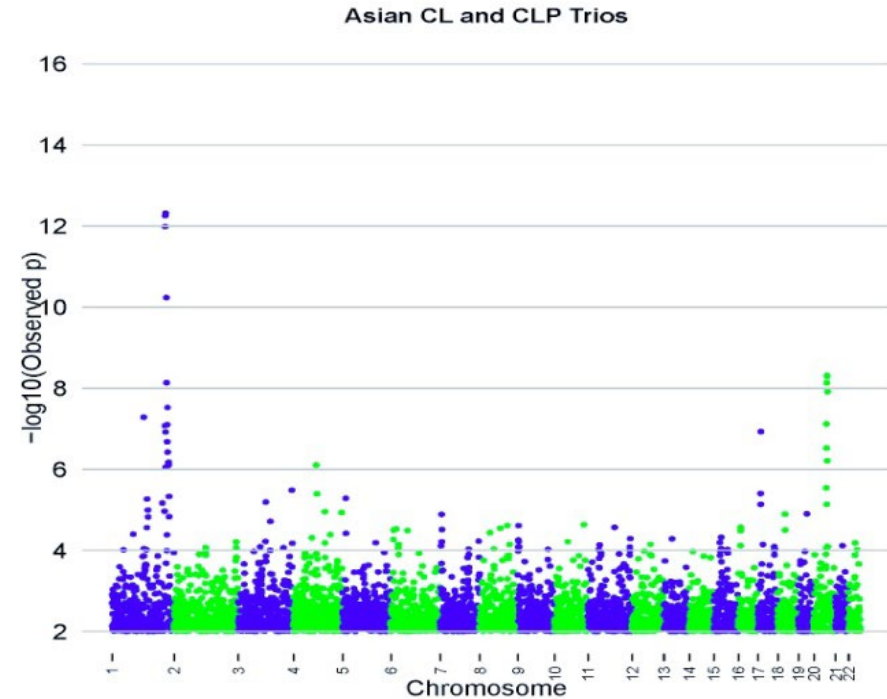
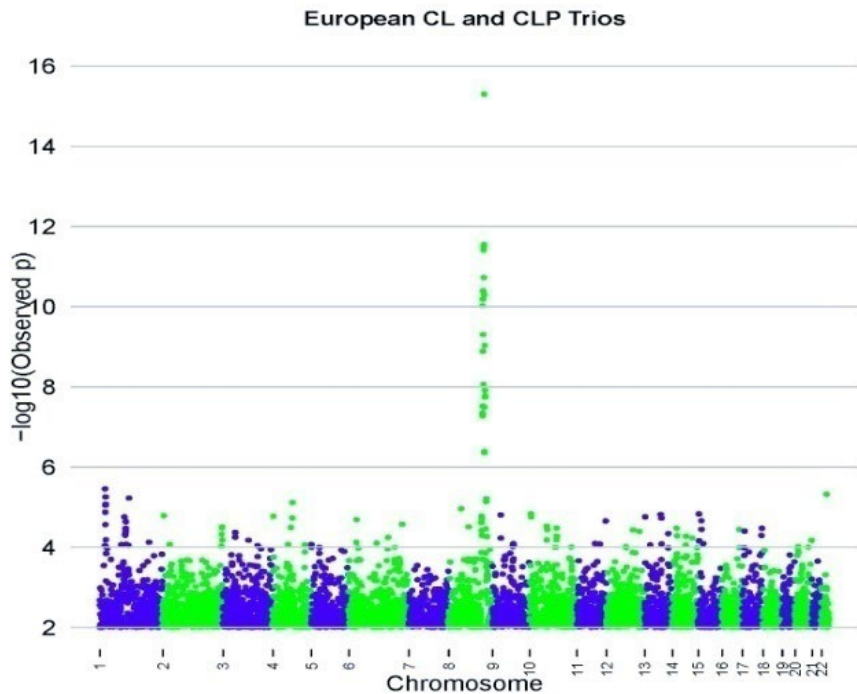
Azeez Butali✉, Peter A Mossey, Wasiu L Adeyemo, Mekonen A Eshete, Lord J J Gowans, Tamara D Busch, Deepti Jain, Wenjie Yu, Liu Huan, Cecelia A Laurie ... Show more

Human Molecular Genetics, Volume 28, Issue 6, 15 March 2019, Pages 1038–1051, <https://doi.org/10.1093/hmg/ddy402>

Published: 19 November 2018 Article history

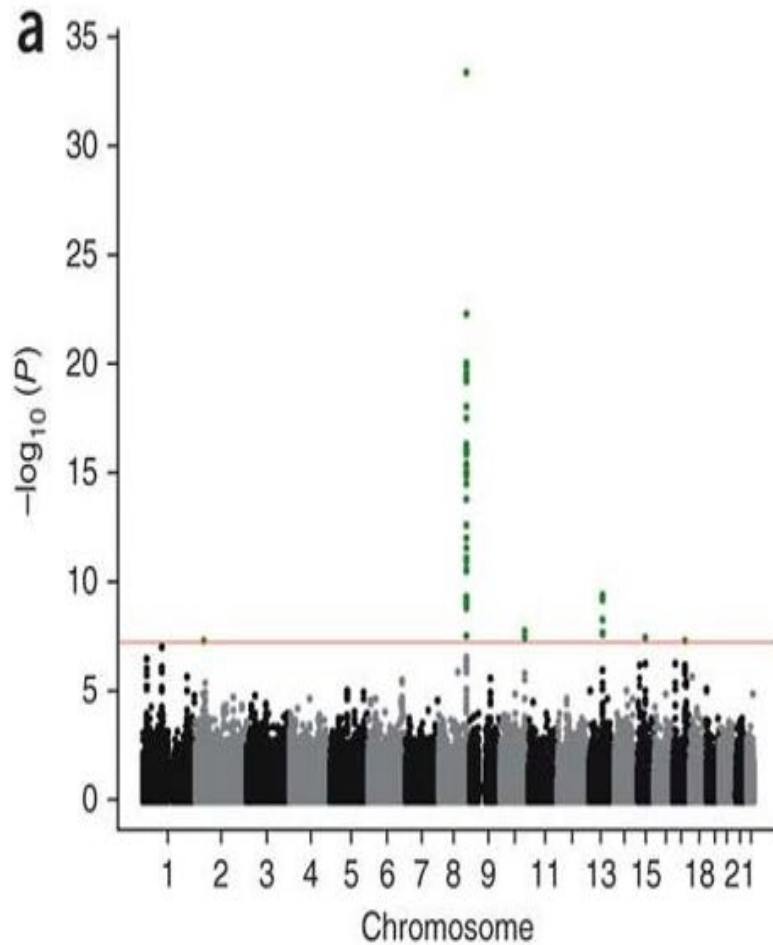
The role of multi-ethnic populations in gene discovery.

Some loci maybe population specific while other loci have associations with multiple populations.

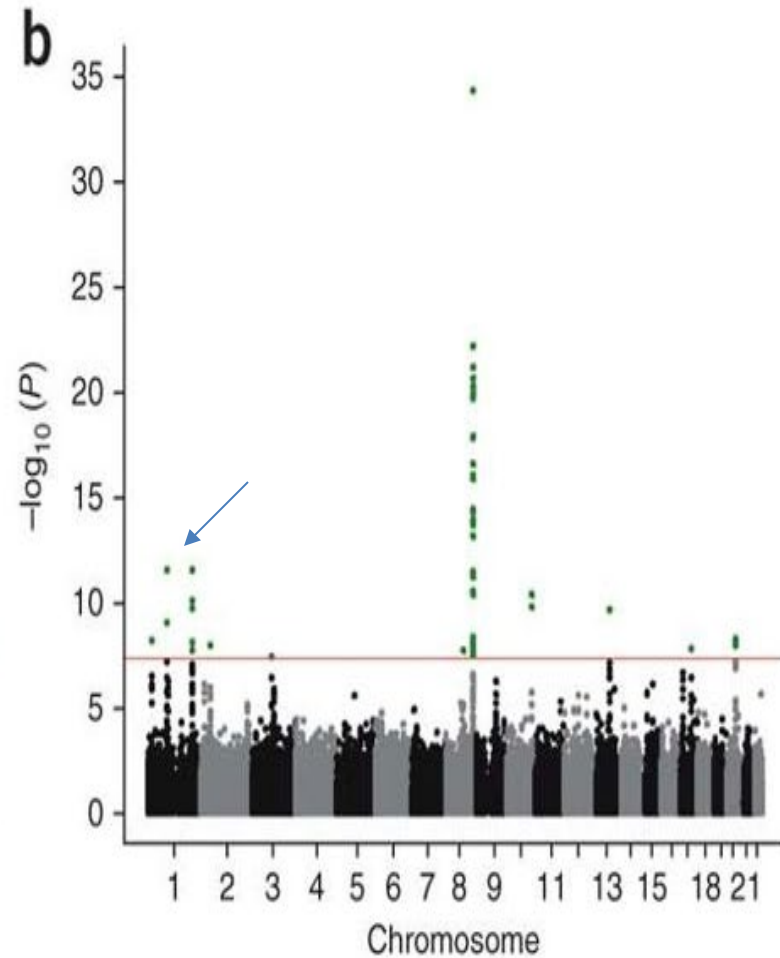


Population differences can be due to differences in allele frequencies.

Low allele frequency significant hits will be missed in single population studies only to turn up later as the sample size increases.



Europeans



Asian and Europeans

Ludwig et al.2012

Strategies for genomics analyses

1. Genome-wide association studies
- 2. Exome sequencing**
3. Whole Genome Sequencing
4. Copy Number Variations

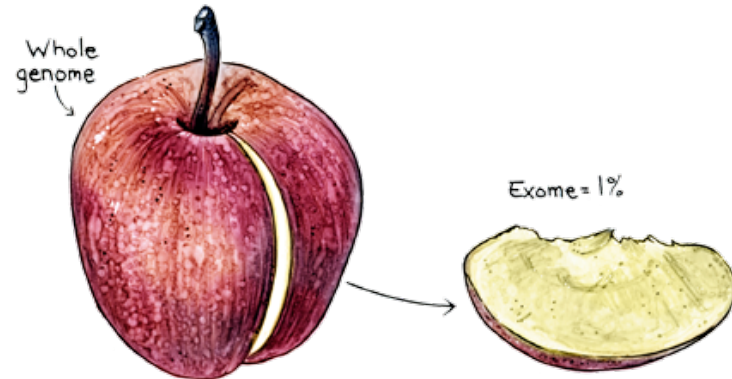
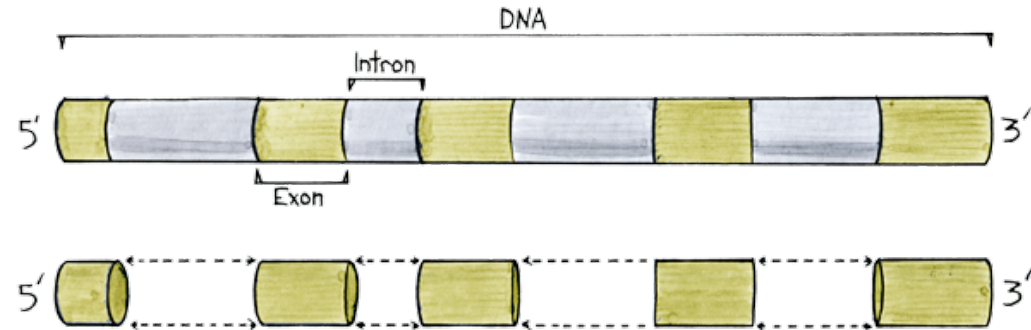
Whole exome studies

Exons are the protein coding regions of the gene.

All the exons in the entire genome are called the exome.

Exome make up about 1% of the entire genome.

About 85% of disease-causing mutations are found in the exome.

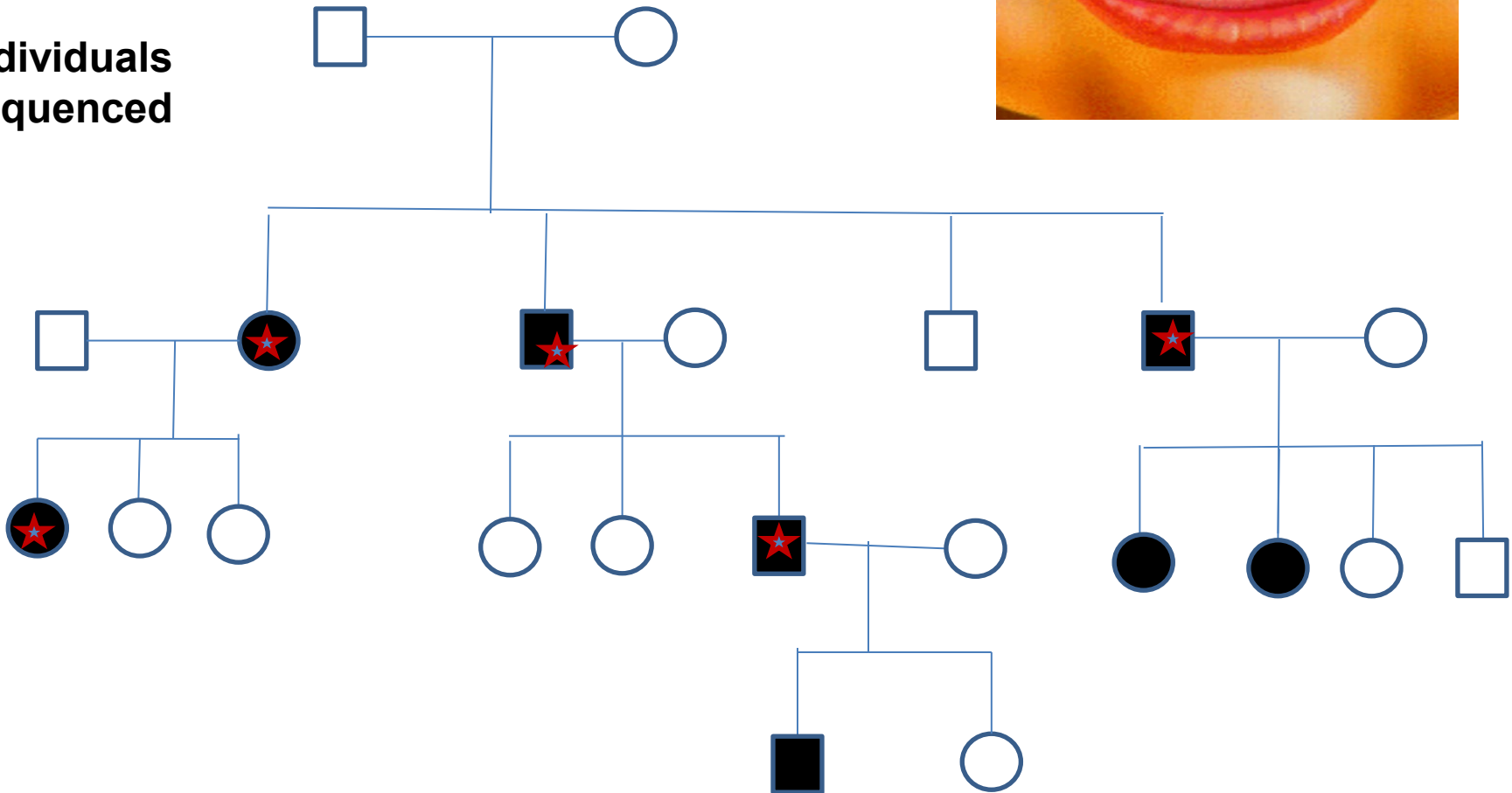


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Exome sequencing of a family with cleft palate only

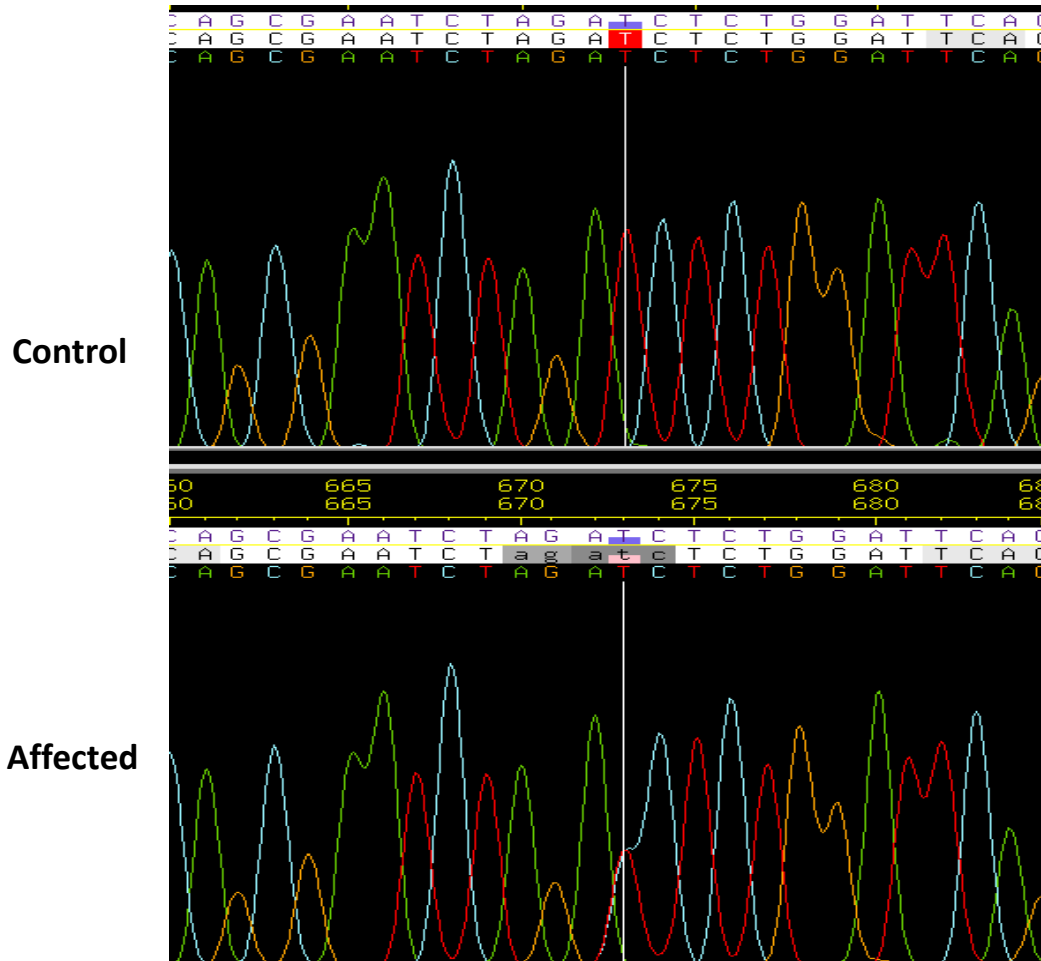


■ ● affected
□ ○ unaffected
★ Individuals sequenced




Mutation p.Ser552Pro segregates in all affected individuals following sanger validation.

No mutation found in unaffected and CEPH Control.



Liu, Butali et al., 2017

Exome sequencing provides additional evidence for the involvement of *ARHGAP29* in Mendelian orofacial clefting and extends the phenotypic spectrum to isolated cleft palate

Huan Liu, Tamara Busch, Steven Eliason, Deepti Anand, Steven Bullard, Lord J.J. Gowans, Nichole Nidey, Aline Petrin, Eno-Abasi Augustine-Akpan, Irfan Saadi, Martine Dunnwald, Salil A. Lachke, Ying Zhu, Adebowale Adeyemo, Brad Amendt, Tony Roscioli, Robert Cornell, Jeffrey Murray, Azeez Butali 




AJHG

Volume 102, Issue 6, 7 June 2018, Pages 1143-1157



Article

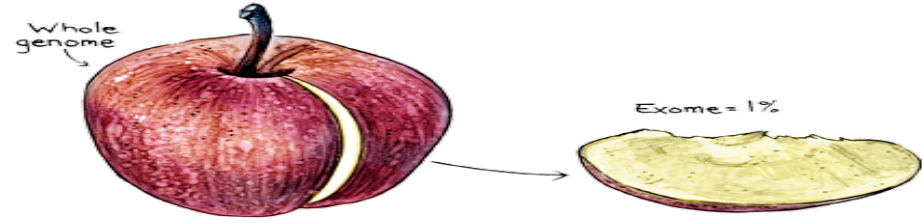
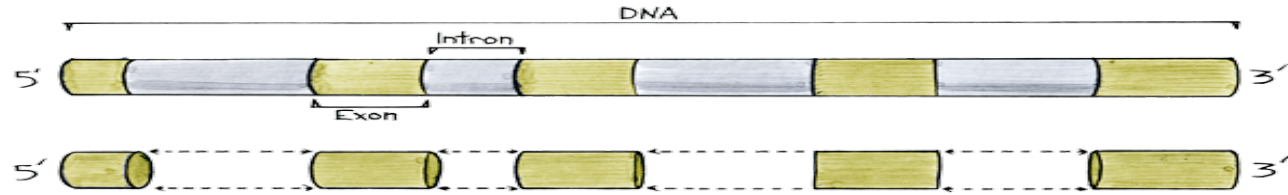
Mutations in the Epithelial Cadherin-p120-Catenin Complex Cause Mendelian Non-Syndromic Cleft Lip with or without Cleft Palate

Liza L. Cox^{1, 2, 3}, Timothy C. Cox^{1, 2, 4, 31}  , Lina M. Moreno Uribe⁵, Ying Zhu^{6, 7}, Chika T. Richter⁵, Nichole Nidey⁸, Jennifer M. Standley⁸, Mei Deng⁹, Elizabeth Blue¹⁰, Jessica X. Chong¹¹, Yueqin Yang¹², Russ P. Carstens^{12, 13}, Deepti Anand¹⁴, Salil A. Lachke¹⁴, Joshua D. Smith¹⁵, Michael O. Dorschner^{16, 17}, Bruce Bedell⁸, Edwin Kirk^{6, 18}, Anne V. Hing^{1, 19}, Hanka Venselaar²⁰, Luz C. Valencia-Ramirez²¹, Michael J. Bamshad^{11, 15}, Ian A. Glass^{9, 11}, Jonathan A. Cooper³, Eric Haan^{22, 23}, Deborah A. Nickerson¹⁵, Hans van Bokhoven^{24, 25}, Huiqing Zhou^{24, 26}, Katy N. Krahn²⁷, Michael F. Buckley⁶, Jeffrey C. Murray⁸, Andrew C. Lidral²⁸, Tony Roscioli^{18, 29, 30, 31}  

Strategies for genomics analyses

1. Genome-wide association studies
2. Exome sequencing
- 3. Whole Genome Sequencing**
4. Copy Number Variations

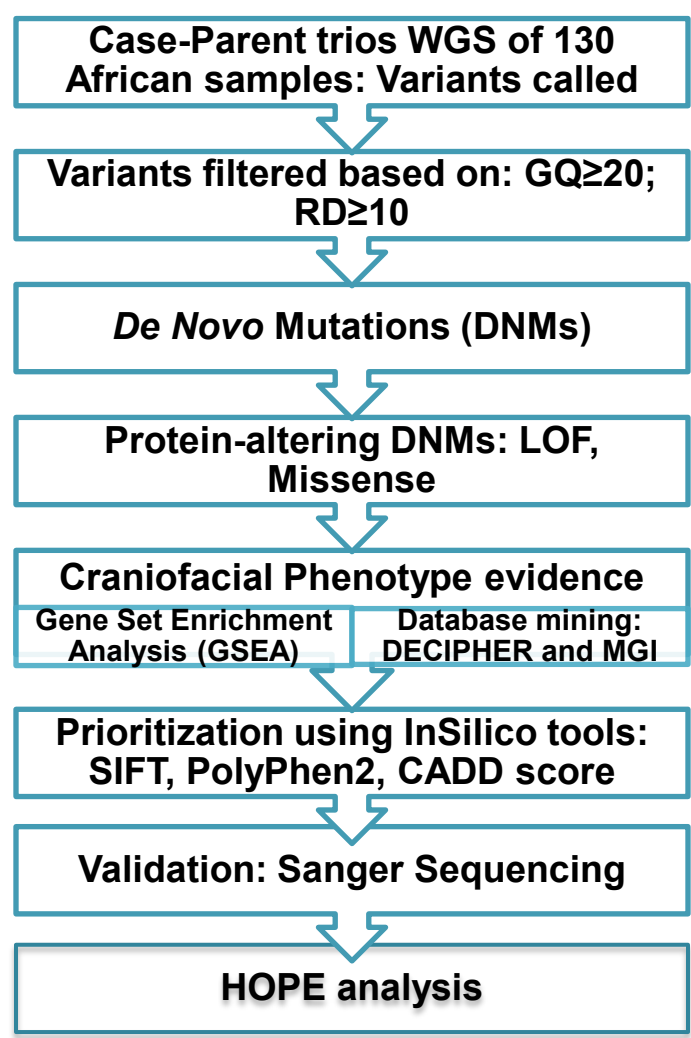
The genome contains the coding region called the **exome** and non-coding region that harbors regulatory elements that affects gene expression.



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De Novo mutations contribute to the risk of sporadic non-syndromic clefts in an African cohort.



De novo loss of function and missense variants

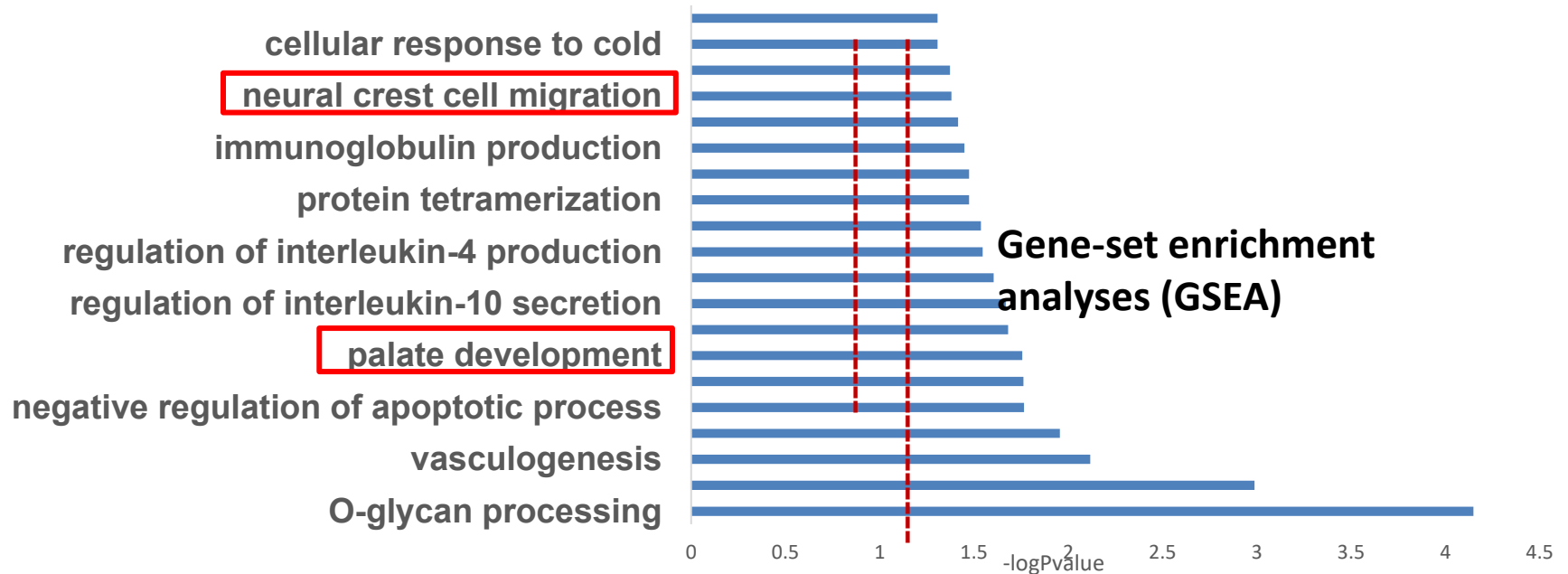
Chromosome Location	Genes	Sequence Ontology	HGVS p.	MGI	CADD Score	SIFT	PolyPhen 2
2:178738241	<i>TTN</i>	LOF	p.Arg4738Ter	Yes	37	N/A	N/A
17:4887701	<i>MINK1</i>	LOF	p.Arg381Ter	Yes	37	N/A	N/A
4:147875102	<i>ARHGAP10</i>	LOF	p.Arg262Ter	Nil	37	N/A	N/A
12:63802407	<i>TMEM5 (RXYL1)</i>	LOF		Nil	32	N/A	N/A
179563094	<i>ACTL6A</i>	LOF	p.Met1Arg	Nil	30	N/A	N/A
1:12617239	<i>DHRS3</i>	missense	p.Ser37Leu	Yes	25	0.013	0.614
6:158493629	<i>TULP4</i>	missense	p.Ser563Phe	Nil	24.5	0.011	0.951
7:155803204	<i>SHH</i>	missense	p.Ser362Leu	Yes	26.4	0.004	1.0
3:189894226	<i>TP63</i>	missense	p.Ile589Met	Yes	22.5	0.001	0.998
8:144512000	<i>RECQL4</i>	missense	p.Asp1102Tyr	Yes	14.77	0.029	0.940



Waheed Awotoye

*Human Genotypic/Phenotypic information from DECIPHER database (DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources. Firth, H.V. et al., 2009. Am.J.Hum.Genet 84, 524-533 (DOI: [dx.doi.org/10.1016/j.ajhg.2009.03.010](https://doi.org/10.1016/j.ajhg.2009.03.010)))

Biological processes relevant to craniofacial development are significantly enriched within the gene sets bearing the DNMs.



	E10.5 Frontonasal	E11.0 Frontonasal	E11.5 Frontonasal	E10.0 Mandible	E10.5 Mandible	E10.5 Mandible (lateral)	E10.5 Mandible (medial)	E11.0 Mandible	E11.5a Mandible	E11.5b Mandible	E11.5 Mandible (medial)	E10.5 Maxilla	E11.0 Maxilla	E11.5 Maxilla	E13.5a Palate	E13.5b Palate	E14.5a Palate	E14.5b Palate	E14.5c Palate	P0 Palate
<i>Acan</i>	84.2	75.2	88.6	64.9	84.4	66.1	59.6	70.9	76.7	82.0	92.5	80.0	73.6	82.7	95.5	99.2	184.8	98.4	224.9	125.8
<i>Act16a</i>	2059.0	2286.8	1976.1	3464.7	1918.2	2578.5	3039.4	2330.2	3285.8	1768.0	2621.8	1820.3	2212.0	2329.0	2096.7	1941.4	1281.1	1581.7	1419.8	956.3
<i>Arhgap10</i>	165.1	138.6	156.6	234.3	134.4	172.6	160.4	143.3	87.0	113.4	115.7	142.2	138.5	138.0	65.3	83.1	91.8	107.0	128.6	83.0
<i>Dhrs3</i>	225.8	289.1	267.9	182.8	160.6	146.0	324.1	219.4	293.4	222.1	438.3	220.5	287.3	236.3	333.7	308.8	340.9	329.3	317.2	1057.8
<i>Fkbp10</i>	295.1	423.6	519.5	522.0	355.2	967.1	1232.4	614.5	870.1	662.6	1318.5	426.9	541.5	613.5	1501.4	940.5	2912.2	2557.9	2736.0	1334.3
<i>Krnl2d</i>	267.2	483.8	461.2	658.1	227.1	730.4	980.4	521.5	626.0	501.4	1034.7	296.3	483.7	541.2	753.6	1027.4	865.3	1382.3	751.8	760.0
<i>Mink1</i>	689.4	563.9	643.0	466.8	702.5	527.8	462.8	606.4	378.1	625.0	495.9	781.8	589.9	548.8	383.8	377.1	465.5	435.8	361.6	439.6
<i>Recq4</i>	159.3	172.6	149.5	267.5	133.5	305.4	301.8	197.8	196.9	134.5	215.1	146.1	179.4	179.3	151.7	139.7	110.6	124.6	120.3	101.6
<i>Shh</i>	2296.4	1277.0	986.5	1054.3	3647.9	499.5	1828.0	1397.6	870.9	1897.0	1584.6	2866.6	1578.7	849.0	1743.1	1377.8	886.4	1491.9	1307.3	878.3
<i>Rxyll1 (Tmem5)</i>	429.2	563.0	413.9	1075.5	432.7	763.4	752.2	503.5	843.2	456.9	693.2	379.6	525.8	480.3	1102.3	892.9	819.5	936.4	1139.6	964.3
<i>Trp63 (Trp63)</i>	390.8	491.7	437.0	586.5	499.4	437.5	818.8	554.9	1165.9	458.3	461.6	516.0	728.0	734.6	1525.9	1805.3	673.9	916.2	899.1	204.9
<i>Tn</i>	86.3	66.5	58.4	42.1	94.3	51.1	34.2	93.2	50.6	157.3	677.6	85.7	94.4	52.3	382.6	779.6	1199.1	1486.4	391.2	8512.3

Using the Systems tool for craniofacial expression-based gene discovery (SysFACE), we identified more genes that contribute to the development of lip and palate in mice.

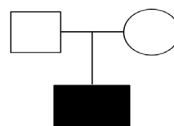
Salil Lachke
 University of Daleware



We interrogated the non-coding regulatory regions of the entire genome to identify the contributions of non-coding variants in the etiology of nsOFC in African populations.



Emmanuel Aladenika



Numbers of enhancers at each step

Numbers of filtered variants at each step

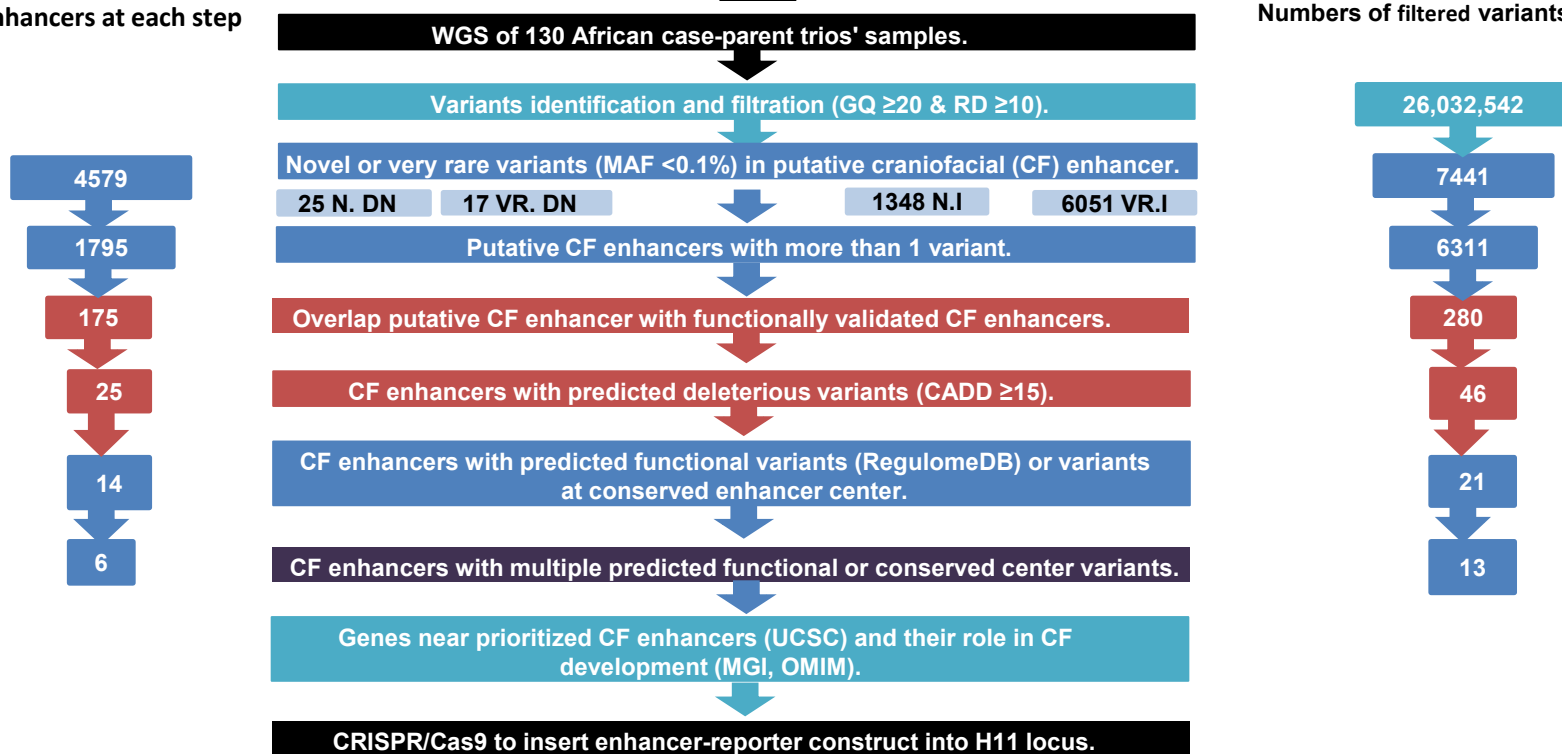


Figure: Data filtration pipeline to identify rare and novel deleterious variants in craniofacial enhancers, identification of craniofacial genes and validation of variants. Flanked on the left and right by the numbers of enhancers and variants at each step, respectively.

N= Novel VR: Very rare NI= Novel Inherited VRI: Very rare inherited

Table : Six craniofacial enhancers each contain at least 2 deleterious variants that are either predicted to be functional or located in conserved enhancer peak regions.

Putative craniofacial enhancers	VISTA enhancer elements	Variants	Ref/Alt	MAF (Africans)	CADD scores	RegulomeD B	PhyloP scores	Middle of peak	Proximal CF gene
chr1:87329509-87331054	chr1:87329513-87331054	1:87330082	G/C	Novel	19.73	4	9.411	Yes	HS2ST1
		1:87330085	C/T	0.00007517	19.52	4	9.349	Yes	
		1:87330628	G/A	0.0002416	17.8	2b	5.267	No	
chr20:7516128-7517759	chr20:7513760-7518083	20:7516992	C/T	0.0001936	18.59	4	4.539	Yes	BMP2
		20:7516913	A/T	Novel	22.2	4	6.469	Yes	
chr14:53571107-53572814	chr14:53569235-53574178	14:53571837	C/T	0.00007242	17.14	5	9.376	Yes	BMP4
		14:53572496	A/T	Novel	16.98	2b	2.855	No	
chr8:38552867-38554293	chr8:38552508-38554672	8:38553746	A/T	0.0003376	21.3	4	2.963	Yes	FGFR1
		8:38553722	C/T	Novel	19.56	4	7.538	Yes	
chr22:20420035-20425595	chr22:20420771-20424717	22:20422768	A/C	0.00007221	21.6	4	2.942	Yes	RANBP1
		22:20422863	C/T	0.0002654	21	4	5.287	Yes	
chr14:29389697-29390978	chr14:29388723-29391342	14:29390539	T/A	0.00002415	19.63	2b	6.442	No	N
		14:29389775	G/A	0.000004	21.3	5	9.445	Yes	

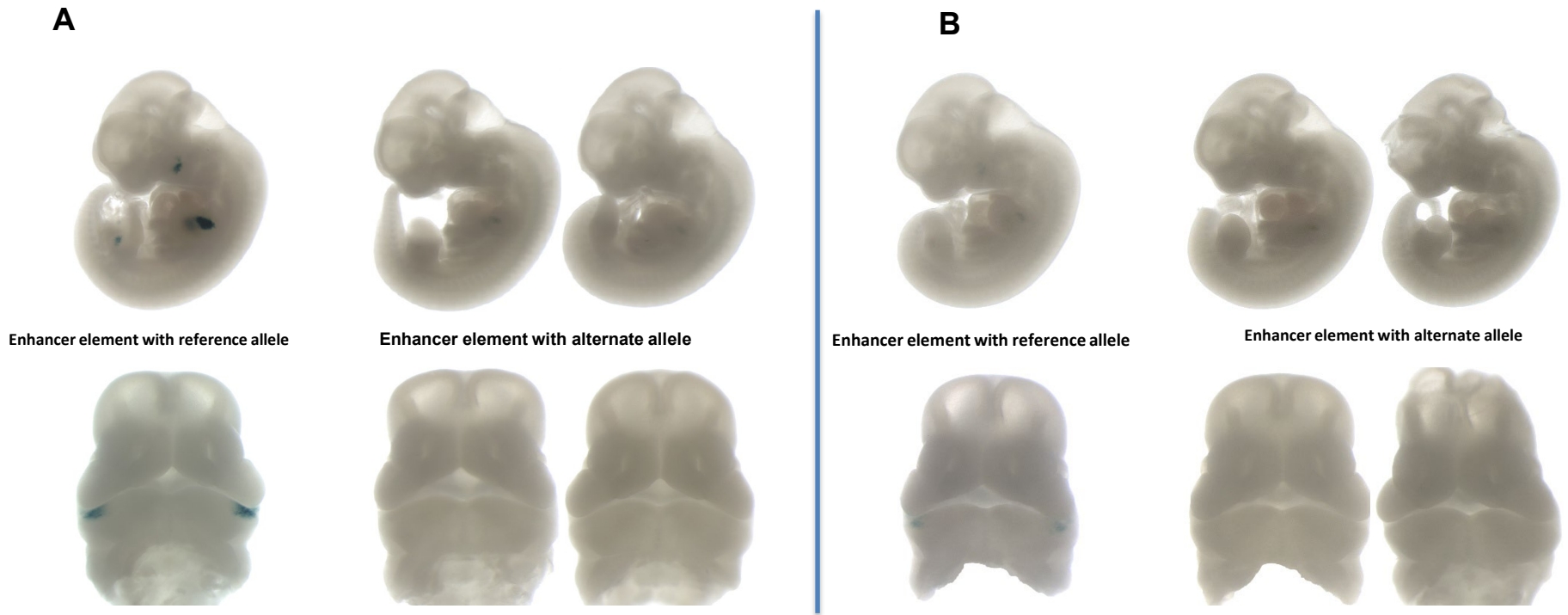


Figure: Chr20:7516128-7517759 enhancer element with reference allele shows lacZ-reporter activity in craniofacial tissue. While same element with alternate allele shows loss of lacZ-reporter activity in mice at e11.5. A. Mice with multiple copies of enhancer-reporter construct at H11 locus (Tandem). B. Mice with single copy of enhancer-reporter construct at H11 locus (Single).

Top row: Sagittal sections of whole body. Bottom row: Coronal section of craniofacial region.

In collaboration with Dr Axel Visel- Lawrence Berkeley National Laboratory.

Strategies for genomics analyses

1. Genome-wide association studies
2. Exome sequencing
3. Whole Genome Sequencing
- 4. Copy Number Variations**

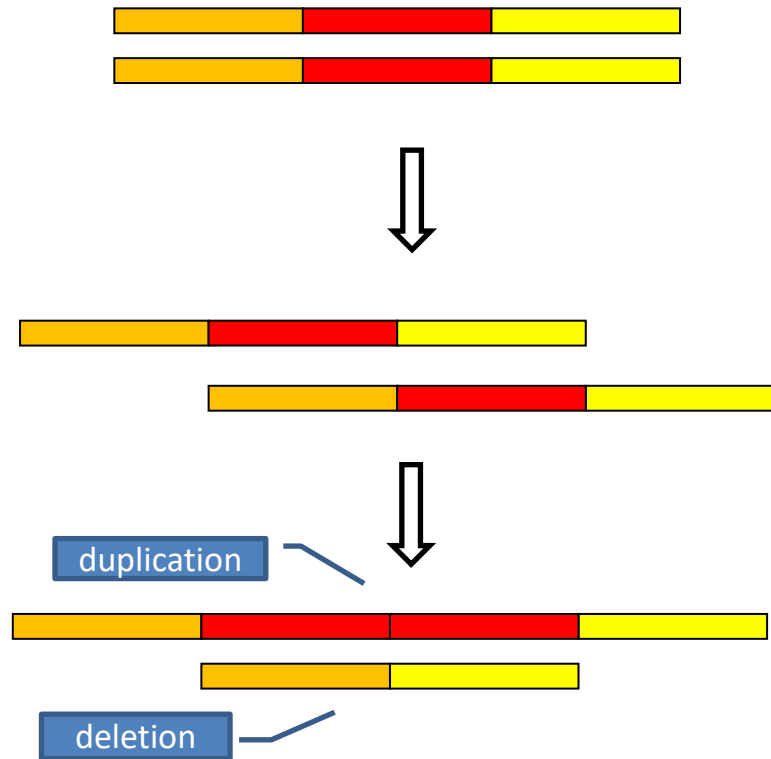
Copy number variations (CNVs)

Deletion of large genomic segments that takes out one or more genes

Segmental Duplications /Amplifications

Non-allelic homologous recombination (NAHR)

- Alignment and cross over between two highly similar DNA sequences



Array-based Comparative Genomic Hybridization (aCGH)

GWAS panel

WGS

Small insertions and deletions

Insertion: actgattggccatgcc



actgattggggccatgcc

Deletions: actgattggccatgcc



actgattccatgcc

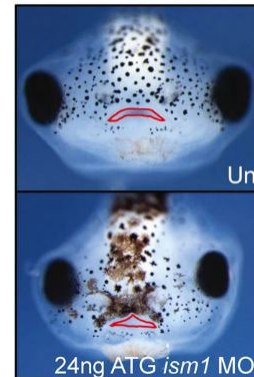
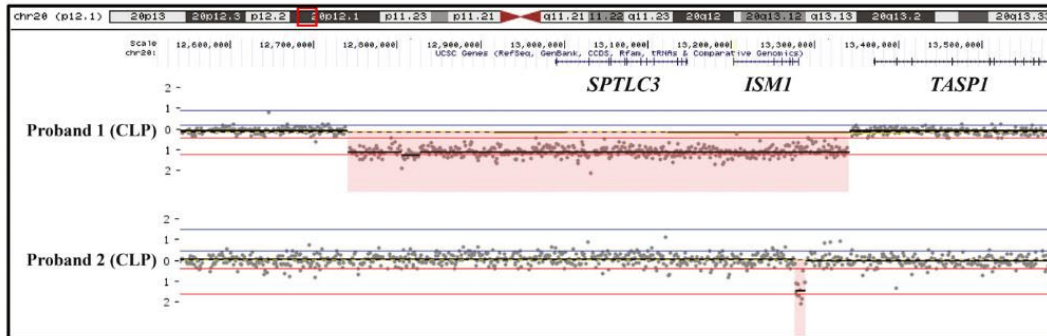
Lansdon et al. 2023. Genome-wide analysis of copy-number variation in humans with cleft lip and/or cleft palate identifies COBLL1, RIC1, and ARHGEF38 as clefting genes.

Rammos et al., 2025. Copy number variants and their implications for developmental and behavioural problems in cleft lip and/or palate.

Array-based Comparative Genomic Hybridization (aCGH)

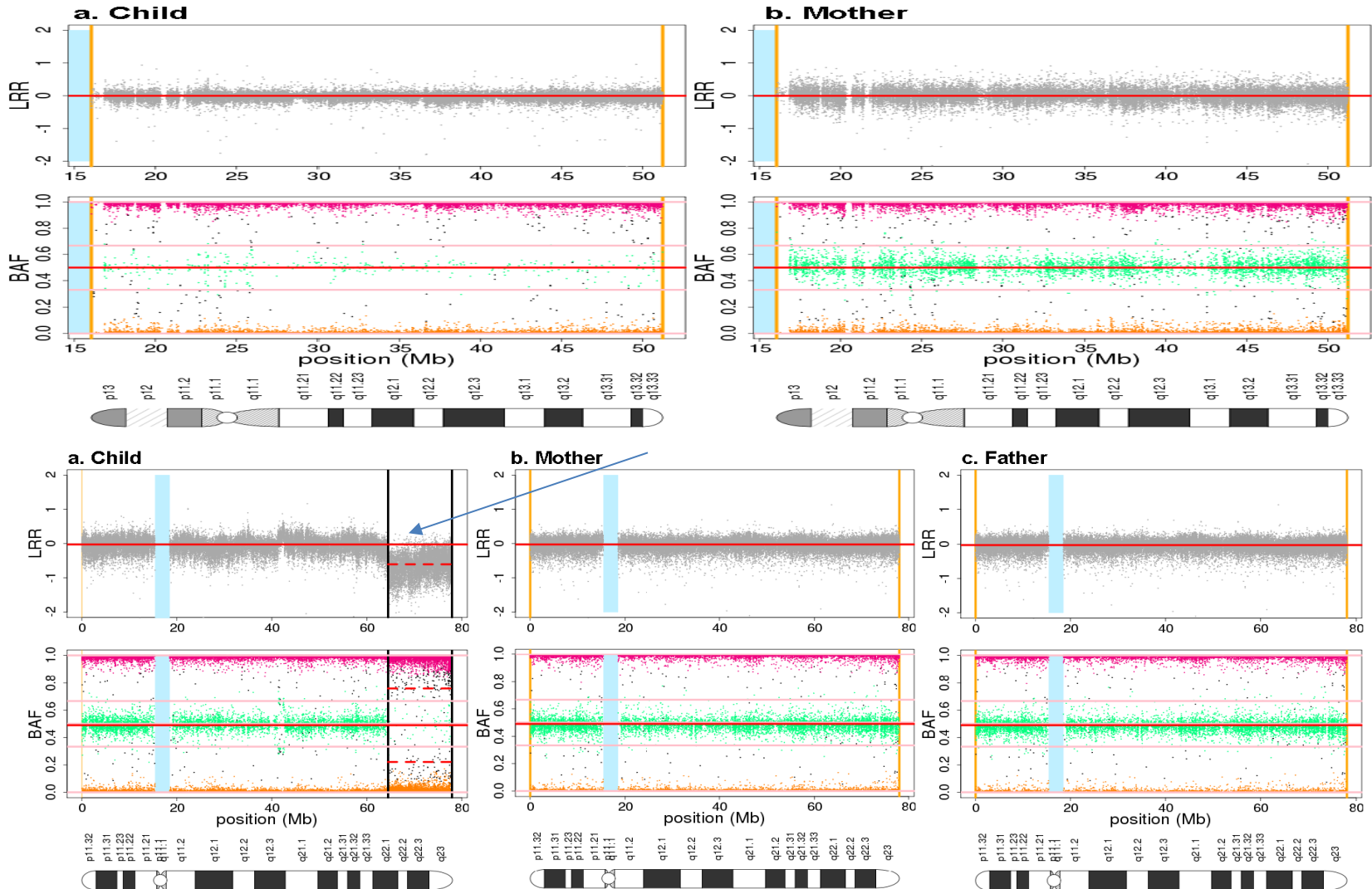
GWAS panel

aCGH
New cleft candidate gene (*ISM1*)
larger deletion – whole gene
smaller deletion – regulatory elements



Adapted from Landson et al. Genetics, Vol. 208,

Identification of paternal uniparental disomy on chromosome 22 and a De-novo deletion on chromosome 18 in individuals with orofacial clefts.



Summary

Genomic analyses of CLP research will largely depend on improvement and advancement in basic , clinical and translational research.

This can be sustained through interdisciplinary and transdisciplinary research and mentoring.

Multi-ethnic populations will accelerate discovery.

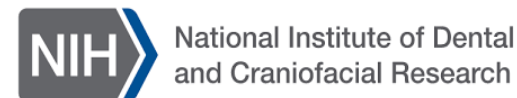
Discovery is the foundation for clinical translation and funding at the discovery level is fundamental.

Acknowledgements

Participating Families, Recruitment staff, data managers and many others



College of Dentistry, University of Iowa



Collaborators

- Jeff C. Murray (University of Iowa)
- Mary Marazita (University of Pittsburgh)
- Martine Dunnwald (University of Iowa)
- Carmen Buxo (University of Puerto Rico)
- Lina Moreno (University of Iowa)
- Robert Cornell (University of Washington)
- Brad Amendt (University of Iowa)
- Eric Van Otterloo (University of Iowa)
- Axel Visel (UC Berkley)
- Erliang Zeng (University of Iowa)
- Aline Petrin (University of Iowa)
- Elizabeth Leslie (Emory)
- Mekonen A. Eshete (Ethiopia)
- Adebowale Adeyemo (NHGRI)
- Wasiu L. Adeyemo (Nigeria)
- Lord J.J Gowans (Ghana)
- Nara Sobreira (John Hopkins University)
- Tim Cox (University of Missouri)
- Karen Liu (Kings College London)
- Steve Twigg (Oxford University)

