From Dysmorphology to Next-Generation Phenotyping

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Society for Birth Defects Research and Prevention's 2020 Virtual 60th Annual Meeting

Conflict of Interest

- Chief Medical Officer for FDNA, company providing Face2Gene app
  - Past: Travel reimbursement and consulting fee
  - Current: Stock options

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Dr. Robert L. Brent, MD, PhD

- World renowned expert on embryonal and fetal radiation exposure
- National and international awards for contributions to the field
- Chairman, Department of Pediatrics at Nemours, Jefferson >30 years

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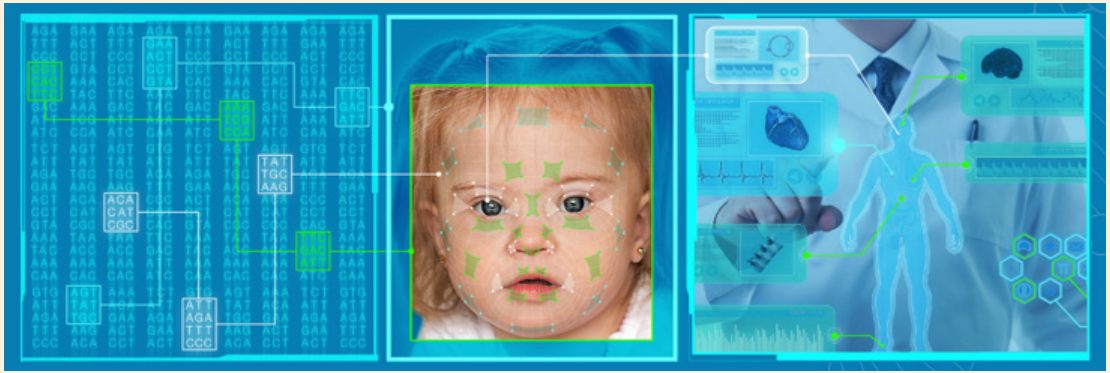
Clinical Genetics:
Integration of genetic and phenotypic information



Next-Generation Sequencing (NGS)

Next-Generation Phenotyping (NGP)

Image from FDNA

A new syndrome: Phenotypic delineation

Aust. paediat. J.
(1977), 13: 114-118

A New Syndrome: Mental Subnormality and Nasal Papillomata

J. M. COSTELLO[1]
Department of Paediatrics, University of Auckland

Costello, J. M. (1977). *Aust. paediat. J.*, **13, 114-118. A New Syndrome: Mental subnormality and nasal papillomata.** Two unrelated children with poor postnatal growth, mental subnormality, similar physical appearance and nasal papillomata present a syndrome for which no cause has been found.

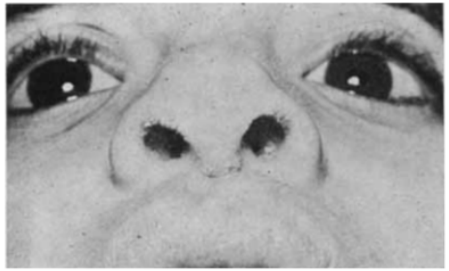


FIGURE 2: Nasal papillomata. Case 1.

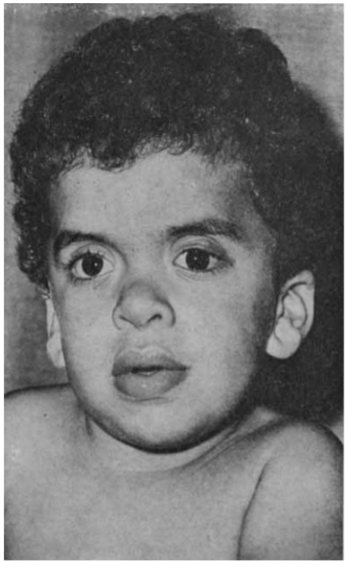


FIGURE 1: Case 1, age 4½ years. Curly hair, depressed nasal bridge, epicanthic folds.

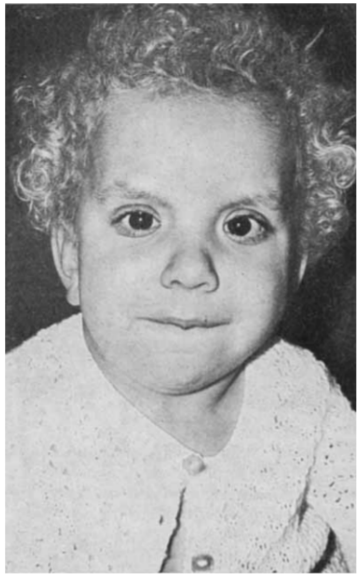


FIGURE 3: Case 2, age 4 years. Curly hair, depressed nasal bridge, epicanthic folds.

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Aust. paediat. J.
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A New Syndrome: Mental Subnormality and Nasal Papillomata

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The author is not aware of any comparable cases reported either before or after these children were described at a meeting in 1971 (Costello, 1971). These children are, therefore, presented as a new syndrome of unknown cause.

TABLE I

	Case 1	Case 2
Gestation	38 weeks	36 weeks
Hydramnios	+	−
Birth weight	3800 gm	3430 gm
Poor sucking	+	+
Poor postnatal growth	+	+
Large head	At birth (now normal)	Since 3½ years (approx)
Short neck	+	+
Curly hair	+	+
Low set ears	+	+
Large ear lobes	+	+
Depressed nasal bridge	+	+
Nasal papillomata	Since 6½ years	Since 2 years
Strabismus	+	+
Epicanthic folds	+	+
Enamel dysplasia	−	+
Thick lips	+	−
Barrel chest	+	+
Short flat hyper-extendible fingers	+	+
Thin nails	+	+
Leg abnormalities	+	+
Loose Integument hands and feet	+	+
High arched palate	+ (at birth)	+ (at birth)
Skin colour	Dark	Olive complexion
Increased carrying angle elbow	+	−
Mental Subnormality	+	+

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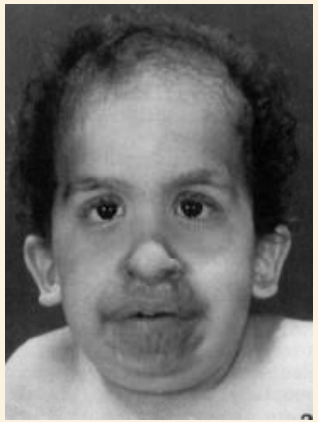
Costello syndrome

Der Kaloustian et al.,
Am J Med Genet 1991

- 3rd patient reported
- Named Costello syndrome
- Noted similarities to Noonan and cardio-facio-cutaneous (CFC)



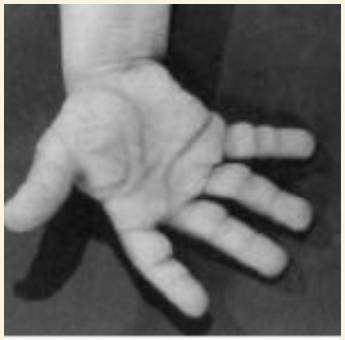


TABLE II. Comparison of Findings in the Noonan, CFC, and Costello Syndromes

	Noonan	CFC	Costello
Poor suck	−	−	+
Poor postnatal growth	+	+	+
"Olive" or "dark" skin color	−	−	+
Ichthyosis	−	+	−
Loose and redundant skin (hands *and* feet)	−	−	+
Hyperkeratotic palms and soles	−	−	+/−
Hyperkeratotic extensor surfaces	−	+	−
Hyperextensible fingers	−	+	+
Relative or absolute macrocephaly at birth or later	−	+	+
Curly hair	+	+	+
Coarse facial features	−	+	+
Strabismus	+/−	+/−	+
Ptosis of eyelids	+	+	+
Epicanthic folds	+	+	+
Apparently low-set ears	+	+	+
Large ear lobes	+	+	+
Depressed nasal bridge	−	+	+
Nasal and perioral papillomata	−	−	+
Thick lips	−	−	+
High-arched palate	+	+	+
Micrognathia	−	+	+
Enamel dysplasia	−	−	+/−
Short neck	+	+/−	+
Webbed neck	+	+/−	−
Pectus excavatum	+	−	−
Pectus carinatum	+	−	+
Barrel chest	−	−	+/−
Heart murmur	+	+	+
Pulmonary stenosis or other congenital heart defect	+	+	−
Undescended testicle	+	+	+
Increased carrying angle	+	+	+
Leg abnormalities	+	+	+/−
Tight Achilles tendon	−	−	+
Skeletal abnormalities	+	+	−
Mental subnormality	+/−	+	+
Seizure disorder	+	+	−
Minor anomalies found in parents	+	−	−

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Costello syndrome

JM Costello, Am J Med Genet 1996: Costello syndrome: Update on the original cases and commentary

TABLE I. Those Manifestations Frequently Seen in Costello Syndrome and Also Frequently Seen in Noonan and/or CFC Syndromes

	Costello Syndrome (out of 16 cases)		Noonan syndrome	CFC syndrome
	Cases	*%*		
Polyhydramnios	6	38	33% [A]	7/24 cases [B]
Postnatal growth deficiency	16	100	50% have height and 43% weight <3rd centile [A]	27/37 [B]
Poor suck/poor feeding	15	94	76% [A]	± [B]
Mental subnormality	16	100	Frequency not well defined. 11% attend special schools [A]	100% [G], 100%[B], 80%[D]
Happy sociable personality	9	56	Usually pleasant [C] Stubborn & irritable [I]	
Epicanthic folds	13*	81	39% [C]	12/14 [B]
Downslanting palpebral fissures	8	50	38% [C]	18/20 [B]
Strabismus	9	56	63% [A]	18/37? [D]
High-arched palate	8	50	51% [E]	16/17 [B] 47% [G]
Depressed nasal bridge	13(?14)	81	+ [A] but high bridge in teenager and adult [C]	20/21 [B]
Wide or long forehead	6	38	Often broad [C]	+ high forehead [F]
Loose skin apart from hands and feet	10	63	Webbing neck 23% [A] Excess nuchal skin 55% [H]	11/12 oedema/redundant skin [B]
Curly hair	13	81	29% [A]	100% sparse, thin, curly [G]
Sparse or short or thin hair	6	38	11% sparse [A]	+ sparse and curly [F]
Nails thin ± deep set ± other	11	69	"Dystrophic" nails [G]	7/18 Dysplastic [B]
Short neck	13	81	+ [A], [G]	
Foot defects	12	75	Talipes equino varus 12% [A]	
Elbow abnormalities	10	63	Cubitus valgus 67% [E]	Cubitus valgus 10% [G]
Cardiomyopathy	6	38	20% [A]	

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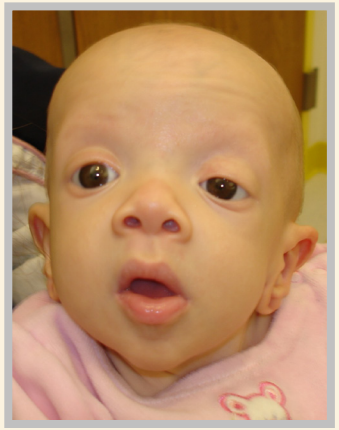
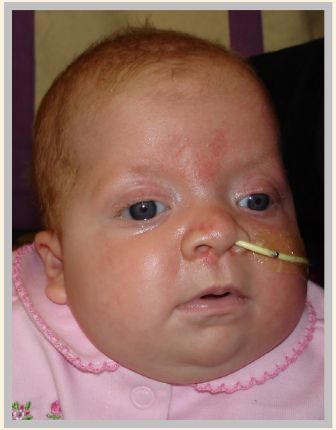
Differential diagnosis

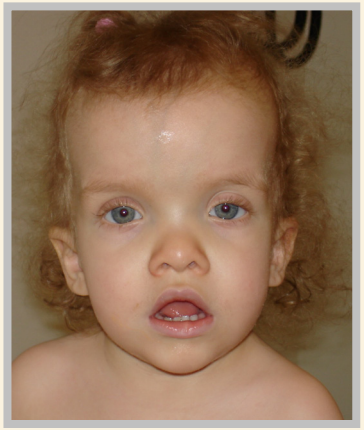
Noonan, Costello and Cardio-Facio-Cutaneous (CFC) syndrome



Noonan



Costello



CFC

Patient images shown with signed consent

Costello syndrome: Further clinical delineation

Cardiac findings in 63% (N=94) Lin et al., 2002

- 30% cardiovascular malformation (pulmonic valve stenosis)
- 34% hypertrophic cardiomyopathy
- 33% atrial tachycardia (chaotic or multifocal)

Tumor predisposition

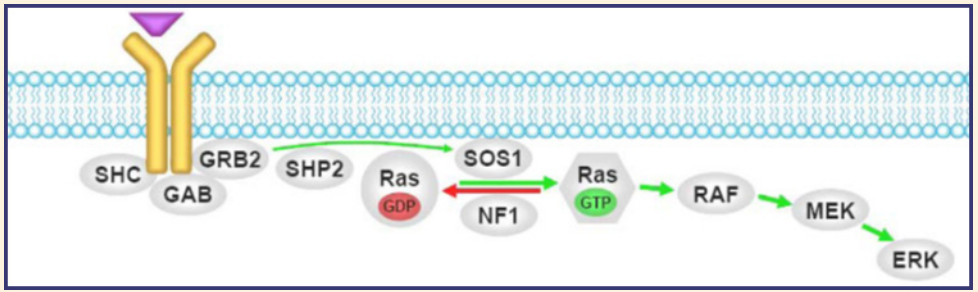
- Kerr et al. 1998: Embryonal rhabdomyosarcoma
  - Gripp et al. 2002: Increased risk for solid tumors (N=17)
    - Embryonal rhabdomyosarcoma; neuroblastoma; bladder cancer
    - Screening protocol
- Kratz et al. 2011: confirmed 15% tumor risk by age 20 years

Cerebellar enlargement and Chiari 1 malformation

- Gripp et al. 2010: 32% Chiari 1 malformation (N=28)

Gene identification: Noonan syndrome

- Linkage to 12q22-qter by Jamieson et al., Nat Genet 1994.
- Heterogeneity noted
- *PTPN11* identified through positional candidate approach by Tartaglia et al., Nat Genet 2001.
- *PTPN11* encodes SHP2, a protein tyrosine phosphatase critical for signal transduction pathways, including the mitogen activated protein kinase (RAS/MAPK) pathway.



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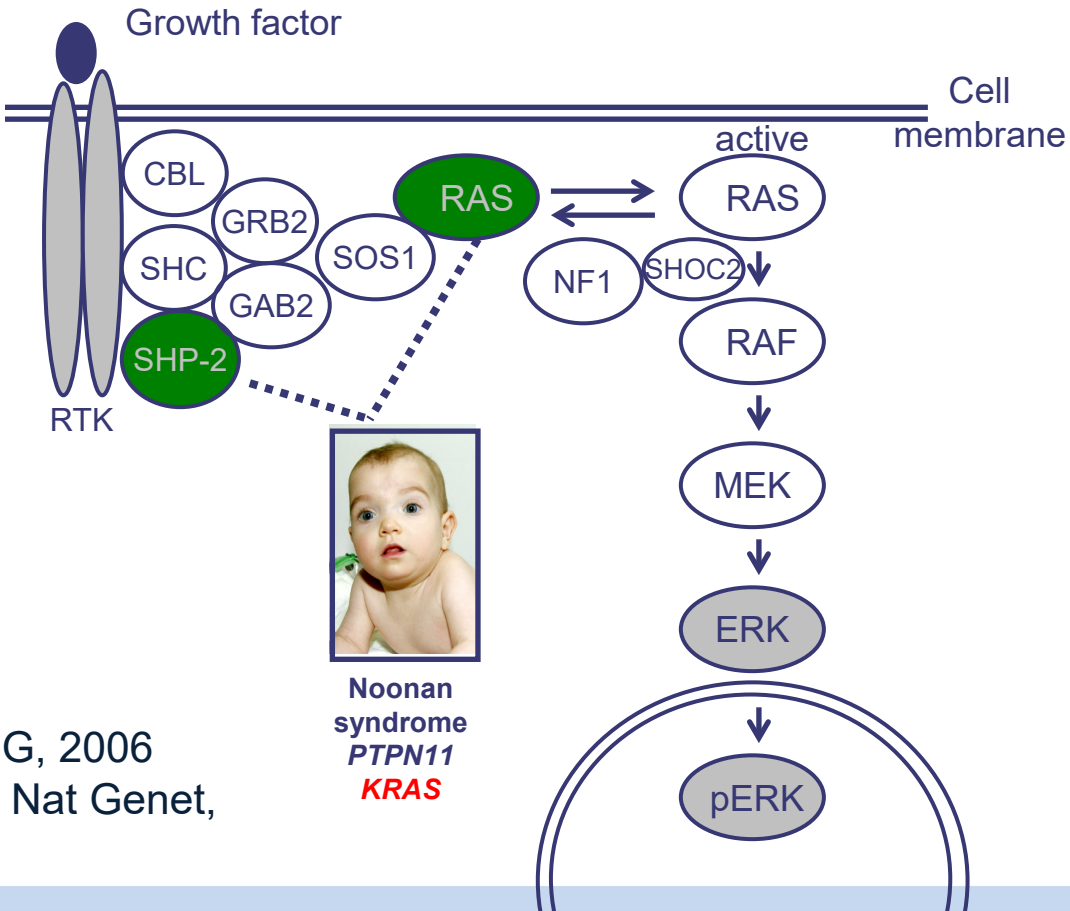
Noonan syndrome: *KRAS* mutations



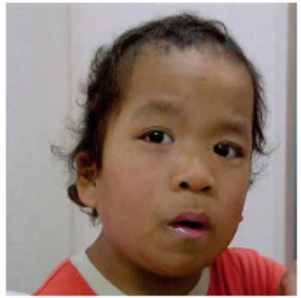
Carta et al., AJHG, 2006
Schubbert et al., Nat Genet, 2006

Gene identification: Costello syndrome

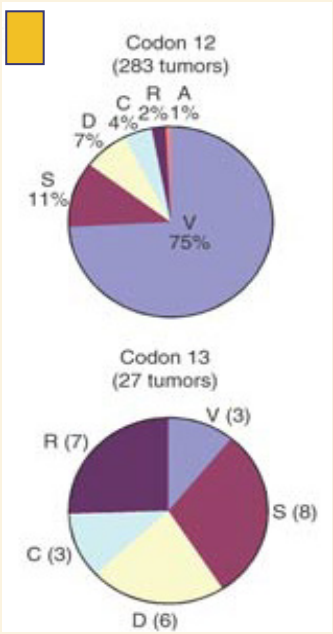
Aoki et al., Nature Genetics 2005

HRAS missense mutations in 12/13 patients

- 10 altered Gly12 (7 Ser, 2 Ala, 1 Val)
- 2 Gly13Asp



Cancer



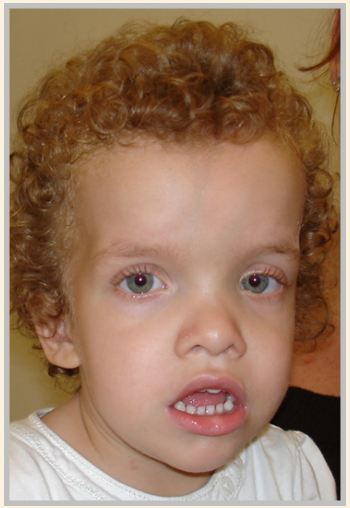
Gene identification: CFC syndrome

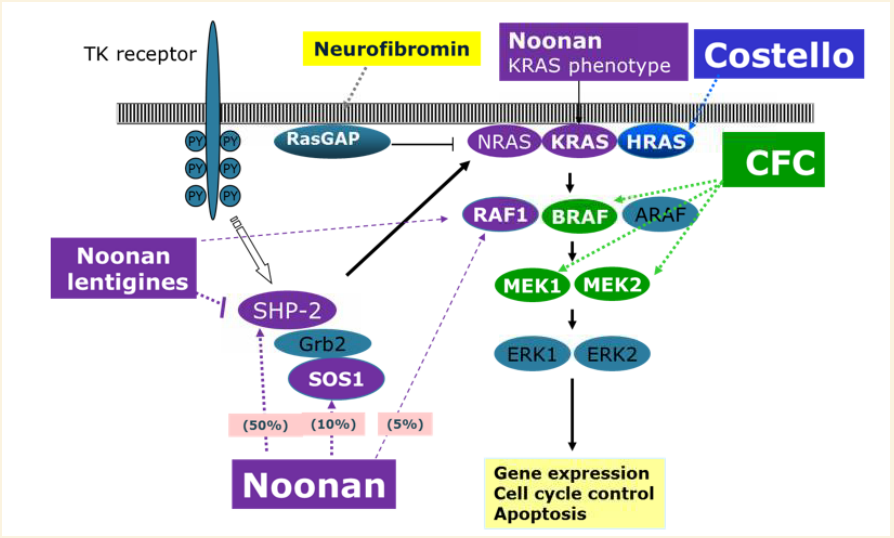
Niihori et al., Nat Genet 2006;
Rodriguez-Viciana et al., Science 2006

- Missense mutations in *BRAF*, *MEK1* or *MEK2*

- RAS/MAPK pathway activation:

Shared mechanism for RASopathies





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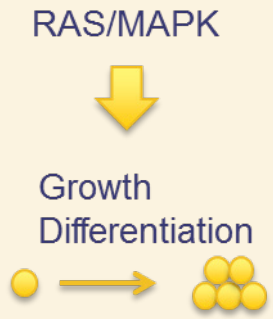
RASopathies

- The RAS/MAPK pathway affects cell division and differentiation.
- Its primary role is likely for embryologic development.
- Early (germline) mutations affect embryologic development and results in syndromic condition.
  Secondary role as proto-oncogenes after somatic mutation.

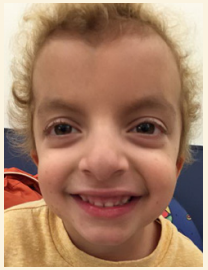


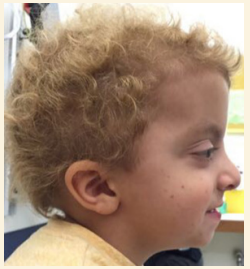
- Delineation from clinical description to molecular cause ~ 30 years
- Numerous genes involved:
  - Testing requires next-generation sequencing approaches
  - Large gene panel or exome analysis

Next-generation sequencing and large databases

Patient 1: Short stature, cryptorchidism, Chiari 1 malformation, macrocephaly, distinctive facial features, slow growing curly hair, developmental delay.
No variant in known RASopathy genes.

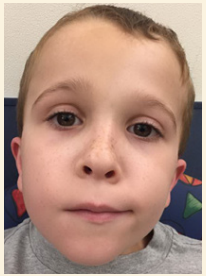
- Exome analysis: de novo *PPP1CB* variant c.146G>C; p.Pro49Arg

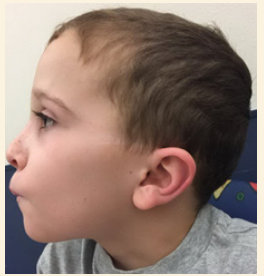




Patient 2: Feeding difficulties, short stature, pectus excavatum, developmental delay.
No variant in known RASopathy genes.

- Exome analysis: de novo *PPP1CB* variant c.166C>G; p.Ala56Pro



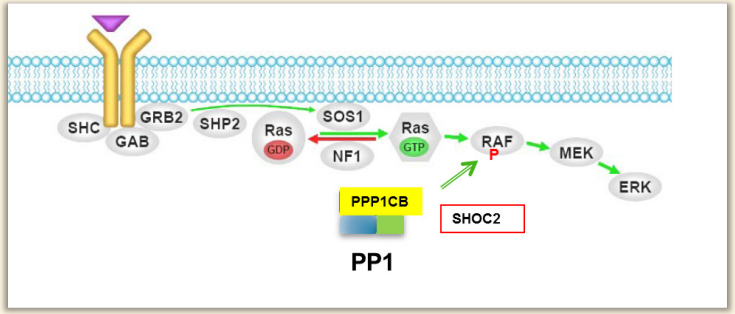


GeneMatcher.org

- Sobreira et al. Human Mut. 2015: GeneMatcher: A matching tool for connecting investigators with an interest in the same gene.

Novel syndrome delineation

- GeneMatcher: 2 additional cases, N=4
- Gripp et al. 2016, Am J Med Genet: Missense mutations in *PPP1CB* cause a RASopathy resembling Noonan syndrome with loose anagen hair.
- OMIM#617506
  Noonan syndrome with loose anagen hair 2 (NSLH2)



Noonan syndrome with loose anagen hair

	PPP1CB	SHOC2
Short stature	9/15	28/31
GH deficiency	2/2	14/17
Macrocephaly (relative)	14/14	29/32
Cardiac	PVS 2/15 ASD 2/15 VSD 2/15	PVS 10/29 Mitral/ tricuspid 8/24
HCM	0/15	6/29
Coarctation/ hypoplastic aortic arch	3/15	1 report
Developmental delay	15/15	5/5
Learning/ performance difficulties	At least 3/4	ADHD common
Hyperpigmentation	4/5	23/31
Slow growing hair	8/9	LAH

Computer technology in clinical genetics

Variant identification and analysis

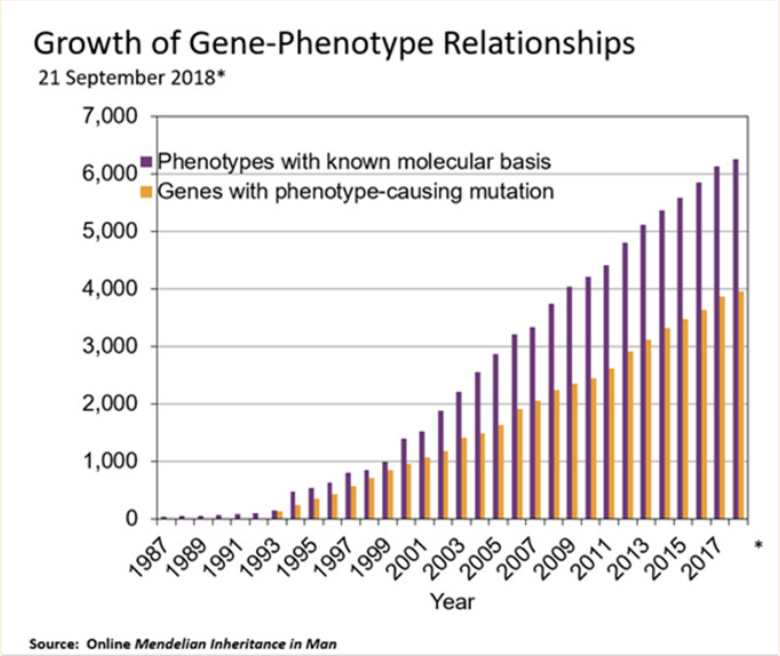
- Next-generation sequencing technology
- Sequencing data analysis through algorithms
- Large databases: gnomAD; ClinVar
- In silico modeling of variant effects on protein product: Mutation Taster, REVEL score
- Matching databases: GeneMatcher

Phenotype analysis

- Human phenotype ontology (HPO) terms
- OMIM database

Next-generation phenotyping

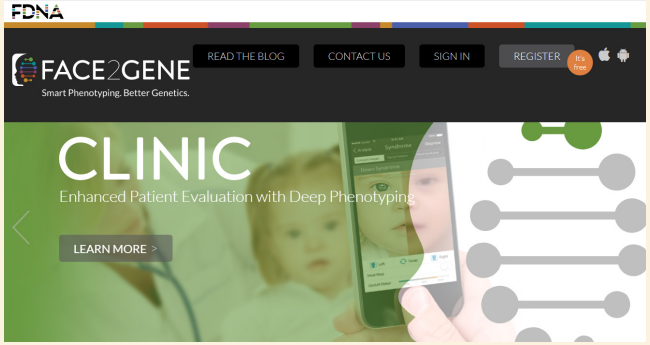
- Next-generation sequencing enabled the identification of novel disease genes and syndromes
- Syndrome identification outpaced the clinical experts' ability to memorize them
- Syndrome recognition is limited by the clinician's individual experience and time
- Syndrome diagnosis is hampered by atypical or mild presentations

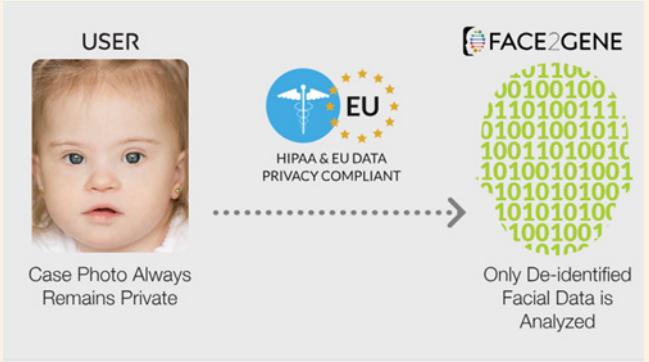


Next-generation phenotyping: DeepGestalt

DeepGestalt is the algorithm used in the Face2Gene app (Face2Gene.com)

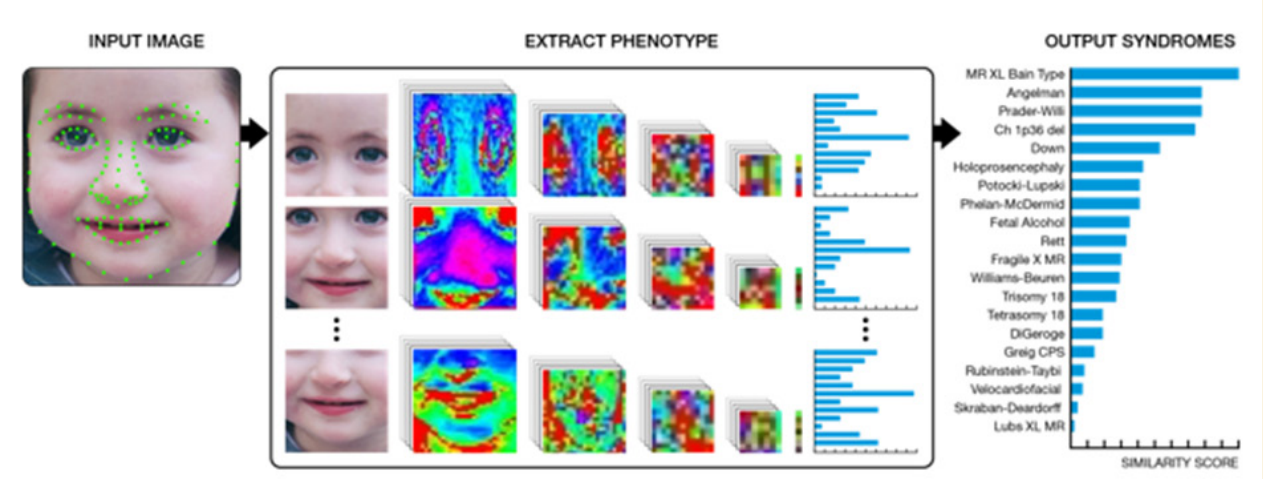
- Unconstrained 2D images
- Deep convolutional neural network (DCNN) approach
- Trained on Casia-WebFace dataset for face recognition
- Fine-tuned to >300 syndromes through training on >17 000 validated patient images
- Community driven: Uploaded images are analyzed in a non-identifiable manner, data is used to further train syndrome recognition





DeepGestalt

- Face recognition, pre-processing
- 130 landmarks placed
- Cropped into predefined facial regions, analyzed through DCNN
- Aggregate results read out as syndrome similarity



DeepGestalt

Gurovich et al., Nature Medicine 2019: Identifying Facial Phenotypes of Genetic Disorders using Deep Learning.

Multiclass analysis

Training set: >200 syndromes, trained on >10,000 diagnosed images

Test set:

Clinical: 502 patient images of cases submitted and solved over time

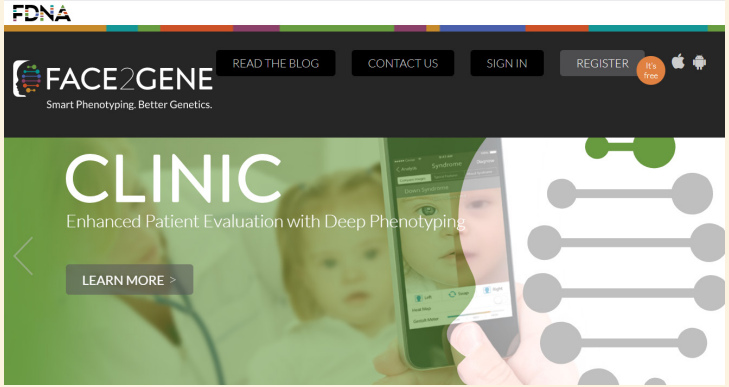
Published: 329 diagnosed patient images from London Medical Database

	Top 10 accuracy	Top 5 accuracy	Top 1 accuracy
Clinical test set	90.6% (CI 88%-93%)	85.4% (CI 82.3%-88.4%)	61.3% (CF 57.2%-65.5%)
Publication test set	89.4% (CI 86%-92.7%)	83.2% (CI 79%-87.2%)	68.7% (CI 63.52-73.55%)

DeepGestalt in clinic today

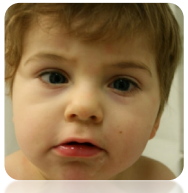
Next generation phenotyping through Face2Gene

- Facial photo taken in clinic
- Additional information provided (“short stature” or “intellectual disability”)
- Image and terms analyzed immediately and matching syndromes suggested
- Targeted testing reduces cost, shortens diagnostic odyssey

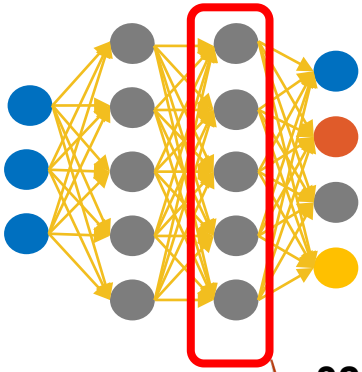


From DeepGestalt to GestaltMatcher

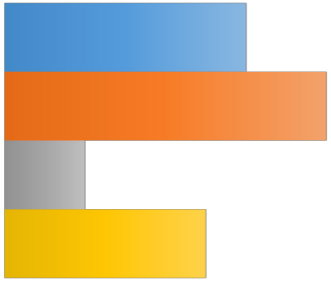
DeepGestalt



Feature encoder

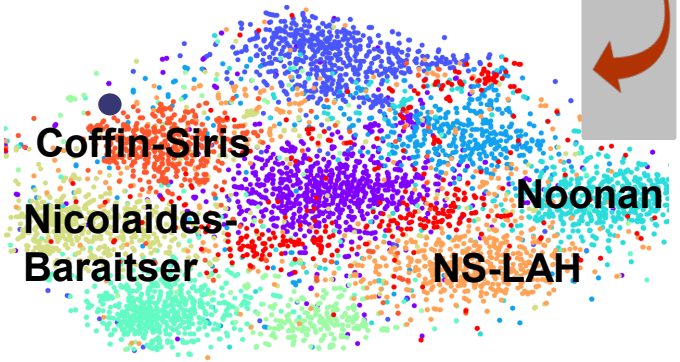


Classifier



Only trained syndromes

320-dimensional facial descriptor fine-tuned by 21k photos over 301 syndromes



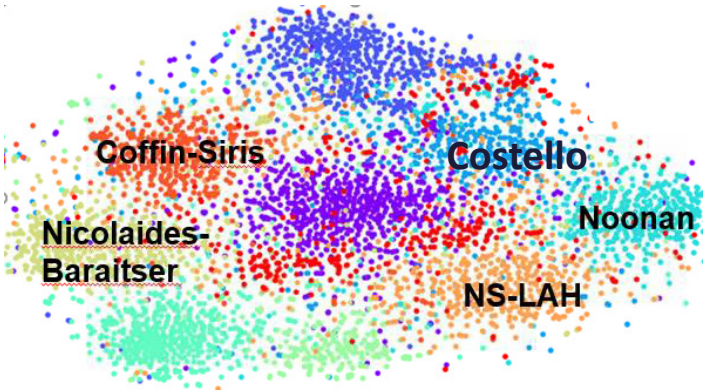
Not restricted to trained syndromes

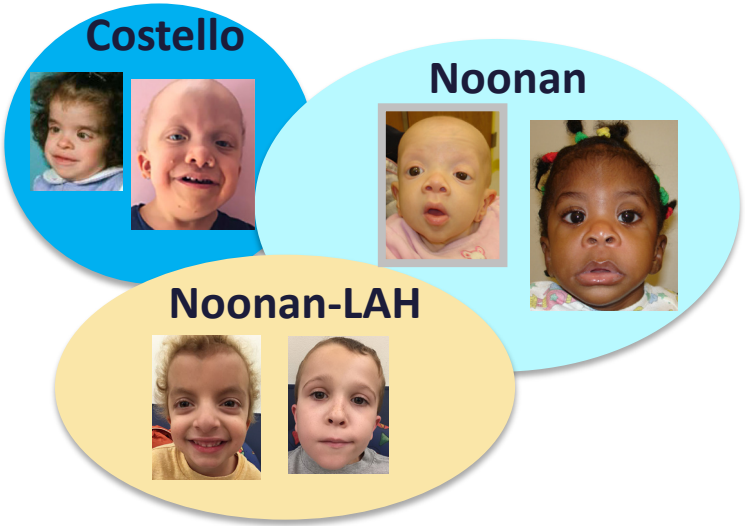
Hsieh et al., ESHG meeting June 2020

From DeepGestalt to GestaltMatcher

- Each patient represented by one dot
- The similarity between two patients is quantified by the cosine distance

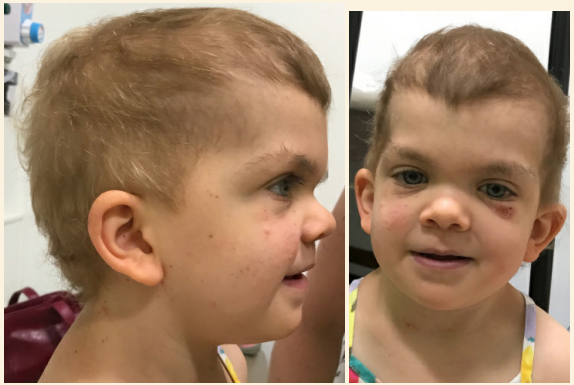
close in space ⟷ **similar phenotype**



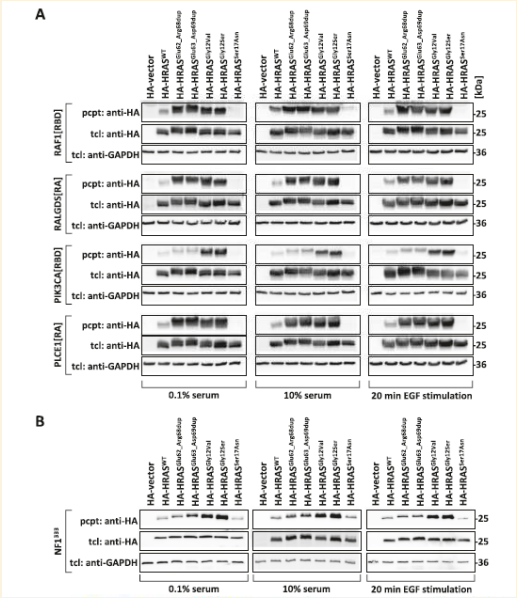


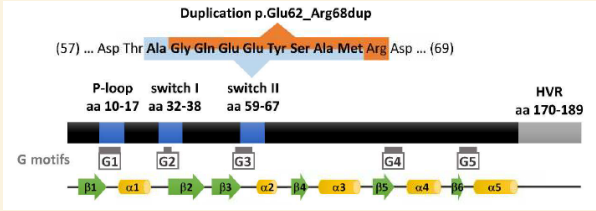
Hsieh et al., ESHG meeting June 2020

An unusual presentation



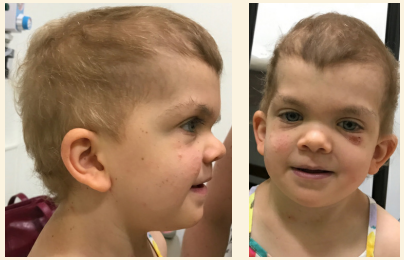
Large for gestational age, macrocephaly
Mild gross motor delay
Slow growing hair, lentigines
Seizure
Chiari malformation, syrinx
Hypertrophic cardiomyopathy
HRAS c.186_206dup, p.(Glu62_Arg68dup)



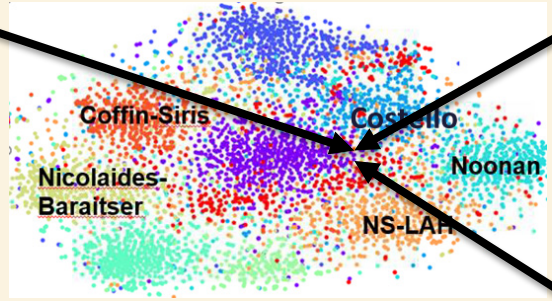


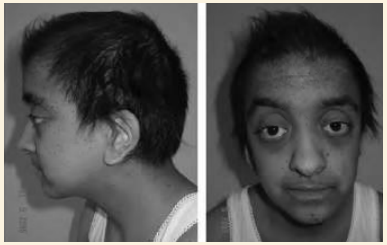
Gripp et al. 2020, Eur J Hum Genet: The novel duplication HRAS c.186_206dup p.(Glu62_Arg68dup): Clinical and functional aspects

Future use of GestaltMatcher?

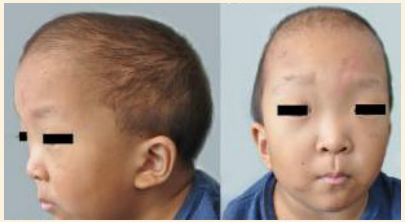


HRAS c.186_206dup,
p.(Glu62_Arg68dup)
Gripp et al. Eur J Hum Genet 2020





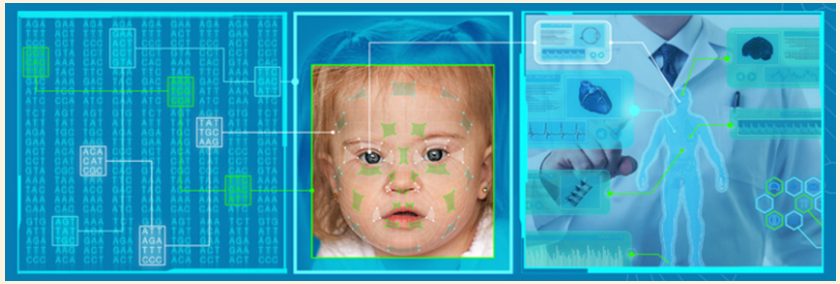
HRAS c.187_207dup,
p.(Glu63_Asp69dup)
Lorenz et al. Hum Mol Genet 2013



HRAS c.187_207dup,
p.(Glu63_Asp69dup)
Xu et al. Clin Exp Derm 2015

Conclusions

- Computer aided sequencing changed genetic testing
- Machine learning will change phenotype analysis
- Together, these technologies will support precision medicine



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- Collaborators- national and international- too many to name

Dr. Robert L. Brent, MD, PhD

Thank you!!!

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Questions?