Accepted Manuscript

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PII: S1098-3600(26)00905-6
DOI: doi:10.1016/j.gim.2026.102587
Reference: GIM 102587

Published in: *Genetics in Medicine*

Received date: 18 August 2025
Revised date: 27 March 2026
Accepted date: 15 April 2026

Cite this article as: Edoh E, Mighton C, Broeren E, Gitau V, Ratliff J, DiStefano M, Gadalla S, Girod A, Hughes M, McCurry H, Patel M, Wilcox EH, Mohammadi M, Paschal C, Spector E, Wilkie AOM, Zackai E, Zarate YA, Graham Jr. JM, Jabs EW, Sanchez-Lara PA, ClinGen Craniofacial Malformations Gene Curation Expert Panel, Evidence-based classification of genes implicated in craniosynostosis disorders using the ClinGen curation framework, *Genetics in Medicine*, doi:10.1016/j.gim.2026.102587

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Evidence-based classification of genes implicated in craniosynostosis disorders using the ClinGen curation framework

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Abstract

PURPOSE

The ClinGen Craniofacial Malformations Gene Curation Expert Panel (Cranio GCEP) was formed in 2020 with an initial target of evaluating genes implicated in craniosynostosis and skull abnormalities. This work summarizes the findings of the Cranio GCEP during its first round of curation, aiming to provide expert guidance for clinical validity of gene-disease relationships in the context of craniofacial malformations.

METHODS

The curation scope of the GCEP was separated into multiple rounds based on frequency of occurrence and uniqueness of associated features. Twelve genes (*EFNB1, ERF, FGFR1, FGFR2, FGFR3, MEGF8, MSX2, POR, RAB23, SKI, TCF12,* and *TWIST1*) were selected, based on review of literature, multi-gene sequencing panels from the Genetic Testing Registry (GTR), and expert input.

RESULTS

On average, there were two disease relationships per gene, ranging from one to six. In total, the Cranio GCEP curated 23 gene-disease pairs. Of these curations, 17 (74%) classifications reached Definitive, 3 (13%) Moderate, and 3 (13%) Limited.

CONCLUSIONS

The classification of gene-disease relationships in round one curation of the Cranio GCEP has contributed to systematically evaluating the validity of gene-disease relationships for craniofacial malformations to establish accurate testing panels and improve patient care. By bringing together content experts to focus on gene curation, the Cranio GCEP facilitates education, new collaboration, and encourages publication of clinical cases in previously discovered genes in order to reflect the broadening spectrum of gene-disease relationships in the craniofacial malformation and craniosynostosis literature.

Keywords: Craniosynostosis, ClinGen, gene-disease validity, craniofacial malformations, genomic medicine

Introduction

Craniofacial development is a complex process regulated by a large number of genes.[1] Bulk and single cell RNAseq research has identified thousands of genes that play a role in this embryologic process, with many of these genes being related to craniofacial congenital anomalies, including conditions like craniosynostosis, cleft lip and palate, and other congenital malformations.[2-4] In 2019, there was a reported number of over 150 craniofacial syndromes, with 25–50/year novel syndromes being characterized and published each year.[1] Craniosynostosis is a predominant feature of craniofacial dysostosis, occurring when the cranial sutures fuse prematurely in infancy, leading to an abnormal skull shape and secondary facial abnormalities.[2,5]

The complexity of craniosynostosis genetics is exemplified by the recognition and extensive literature on *FGFR2*- and *FGFR3*-related syndromes.[6-8] *FGFR2*(HGNC:3689) variants are frequently related to clinically recognizable and differentiated syndromic craniosynostoses such as Crouzon(OMIM:123500), Pfeiffer(OMIM:101600), and Apert syndromes(OMIM:101200). These syndromes display characteristic but variable phenotypic expressivity and multisuture involvement.[7] Distinct and recurrent *FGFR3* (HGNC:3690) variants within specific domains, on the other hand, have been implicated in conditions such as Muenke syndrome(OMIM:602849).[8,9] The clinical overlap between *FGFR*-related craniosynostosis and their variable expressivity complicates genotype-phenotype correlations which emphasize the need for rigorous gene-disease classification efforts. Many genes implicated in the craniofacial dysostosis field benefit from a reappraisal of clinical relevance, with evidence-based assessment focused on thorough and updated literature search and expert validation. This ultimately helps ensure accurate classification of gene-disease relationships with downstream effects in the diagnostic laboratory and clinical setting.

The Clinical Genome Resource (ClinGen) is a National Institutes of Health (NIH)-funded resource that aims to build a central database to define the clinical relevance of genes and variants for use in precision medicine and research.[10] As of May 2025, ClinGen supports 47 active Gene Curation Expert Panels (GCEPs) across different disease areas. ClinGen GCEPs consist of disease-specific international groups of experts that have wide representation of the genetics field, including researchers, clinicians, diagnostic laboratory experts, and ClinGen framework experts. GCEPs have a goal of defining the strength of gene-disease relationships by using a semi-quantitative ClinGen curation framework.[11] This widespread effort implements standardized and transparent evidence-based appraisal of gene-disease relationships across multiple disease areas. The framework is well suited to evaluating genes implicated in monogenic disorders, including craniofacial syndromes. While the framework provides a solid base for

curation, it also allows the flexibility of specification by GCEPs in different disease domains and does evolve over time based on expert input, as evidenced by the versioned standard operating procedure on the ClinGen website (https://clinicalgenome.org/docs/gene-disease-validity-standard-operating-procedure/).

The Craniofacial Malformations (Cranio) Gene Curation Expert Panel (GCEP) was formed with the initial goal to evaluate gene-disease relationships related to craniosynostosis and skull abnormalities. ClinGen curation of case-level and experimental data within the Cranio GCEP involves scoring genomic variant information alongside clinical presentation to determine the strength of a relationship between the gene, genotype and disease. Expert panel-approved gene curations are made public on the ClinGen website (clinicalgenome.org), to ensure open access to data that could impact healthcare systems or practice guidelines.

Methods

Formation of GCEP

The Cranio GCEP was formed in 2020 with an initial group of 21 members, broadly representing the field of craniofacial malformations. Membership included: clinicians experienced in diagnosing craniosynostosis-related disorders; diagnostic laboratory experts with experience in genetic testing of genes within the group's scope; ClinGen framework experts; and staff and volunteer biocurators. The Cranio GCEP currently comprises 32 active members. (https://clinicalgenome.org/affiliation/40059/). Expert guests are invited to attend specific curations if they are authors of key manuscripts and or have expertise in the specific phenotype or gene being reviewed.

Gene selection

The disease scope of the Cranio GCEP includes conditions for which craniofacial malformations are a cardinal feature, and often the presenting feature. The initial focus of the group in round one curation was to evaluate the evidence for genes implicated in craniosynostosis and skull abnormalities, with a focus on potentially clinically distinguishable syndromic conditions due to their prevalence, amount of completed clinical and translational research and the body of medical literature and growing use of molecular testing in the clinical setting.

From an initial list of 145 genes, twelve genes were prioritized for initial curation based on their presence on at least 85% of multigene testing panels in the Gene Testing Registry (GTR),[12] clinical relevance, likelihood of strong evidence for gene-disease

relationships, and on expert consensus combined with review of literature. There was also consideration for the genetic contribution of variants in the gene to the disease.

The initial round of genes included: *EFNB1*(HGNC:3226), *ERF* (HGNC:3444), *FGFR1* (HGNC:3688), *FGFR2, FGFR3* (to be done in tandem with the ClinGen Skeletal Disorders GCEP), *MEGF8* (HGNC:3233)*, MSX2* (HGNC:7392)*, POR* (HGNC:9208*), RAB23* (HGNC:14263)*, SKI* (HGNC:10896)*, TCF12* (HGNC:11623), and *TWIST1* (HGNC:12428).

Precuration process

Due to the heterogeneity of craniosynostosis-related disorders, all genes were precurated using the ClinGen Lumping and Splitting guidelines.[13] Each precuration considered the existing nosological information from the craniosynostosis literature in tandem with the Lumping and Splitting guidelines to determine how many disease assertions there were for each gene within the Cranio GCEP's scope.[14] Other sources crucial to determine existing disease assertions in the precuration process were online gene-disease databases like Online Mendelian Inheritance in Man (OMIM)[15] and Mondo Disease Ontology (MONDO).[16] Decisions to lump or split are based on existing disease assertions, phenotypic variability, differences in inheritance pattern, and differences in molecular mechanism.[13]

Curation process

Following the precuration, each gene-disease relationship was evaluated by a single biocurator using ClinGen's framework as described in ClinGen's Gene Curation Standard Operating Procedure (SOP) Version 7 and 8. This process involved a thorough literature search using sources such as PubMed, GoogleScholar, Web of Science, The Jackson Laboratory (JAX), and Mouse Genome Informatics (MGI). [17-19] Per the SOP, gene curation included consideration of genetic evidence, such as observations of affected probands and variant segregation in families, and experimental evidence, such as gene expression in relevant tissues, cellular assays, and animal models.

The ClinGen Gene Curation working group's framework standardizes the approach to determine the validity for a gene-disease pair. Evidence should total ≥12 points and demonstrate replication over time (more than two publications with convincing evidence over three years after the initial publication) per the SOP to reach a Definitive designation.[11] A Strong classification is reached when a gene-disease pair results in a score equal to or greater than 12 points but has not been replicated over time. As a

specification of the framework, the Cranio GCEP decided to upgrade points (add 0.5 points) in cases with radiographs that show phenotypic specificity of craniosynostosis and impact of the variant (Figure 1). This decision was made due to the importance of phenotypic specificity in the clinical setting and was discussed at length in the monthly expert panel calls.

There were a few curations where our group would score differently due to the unique and clinically distinguished presentation of a disorder (see results section for specific curation challenges).

Gene curation involved scoring variants of interest with some evidence of alteration to gene function and ensured representation of the full spectrum of variation, including recurrent variants, for comprehensive gene curations.

Review by experts

A dual review process was used. Once the primary biocurator finished their curation, the chairs of the GCEP would review the curation and recommend changes to scoring, if necessary. The curation was then presented to the full working group on monthly calls. Initial points and classifications were discussed and modified as needed. Point modifications arose from such discussion as: a case not presenting with typical phenotypes and being downgraded or excluded from the curation; a disease being caused by a single recurrent variant and scored multiple times; the methodology used to identify a variant was not rigorous enough, or there is inconsistent segregation of the variant with the disease phenotypes in a familial case to justify full points.

Summary and publication

Evidence summaries were written after the call for each curation based on the updated score and discussion from the experts on the monthly calls using ClinGen's standardized evidence summary text as a guide. These evidence summaries are marked with the GCEP approval date and are publicly available on ClinGen website, along with all individual pieces of evidence used for scoring each gene-disease relationship (https://search.clinicalgenome.org/kb/affiliate/10059?page=1&size=25&search=). ClinGen curations are updated periodically, to find the most current information please visit clinicalgenome.org.

Results

Precuration results

On average, there were two disease assertions per gene, ranging from one (*EFNB1, ERF, MEGF8, POR, RAB23, SKI, TCF12*) to six unique and clinically distinguished disease assertions (*FGFR2*).

Curation of the disease assertions for the *FGFR3* gene revealed extensive overlap between the Cranio GCEP and Skeletal Disorders GCEP's scope, and this gene's disease assertions were divided accordingly between the two GCEPs. The Cranio GCEP curated *FGFR3*-related Muenke syndrome and *FGFR3*-related Crouzon syndrome with acanthosis nigricans (OMIM:612247), while the Skeletal Disorders GCEP curated *FGFR3* for *FGFR3*-related achondroplasia (OMIM:100800), *FGFR3*-related thanatophoric dysplasia (curation pending), *FGFR3*-related camptodactyly-tall stature-scoliosis-hearing loss syndrome (OMIM:610474), *FGFR3*-related hypochondroplasia(OMIM:146000), and *FGFR3*-related severe achondroplasia-developmental delay-acanthosis nigricans (SADDAN) syndrome(OMIM:616482).

Following precuration, 7 genes were curated for a single disease entity, and 5 were curated for 2 or more disease entities (Table 1). One example of a gene for which phenotypes were lumped was the *POR* gene. There is variability reported in the literature, with some cases with pathogenic *POR* variants having isolated craniofacial phenotypes and others with isolated genital anomalies and abnormal steroidogenesis.[20] At the time of curation, disease ontology websites also differed in their disease terminology: OMIM and MONDO had an assertion for Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis(OMIM: 207410), whereas Orphanet had an assertion for Antley-Bixler syndrome.[21] After careful review of the literature, we choose the lumped term "Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis" due to the spectrum of phenotypes found in individuals in the curation. The cases scored in the curation all exhibited craniofacial phenotypes, genital anomalies, and defective steroidogenesis in combination while the evidence summary displayed on the ClinGen website described the phenotypic spectrum and the possibility for cases to exhibit isolated craniofacial or genital features.[6,22,23]

Curation results

The Craniofacial Malformations GCEP curated 12 genes and 23 gene-disease relationships, which resulted in classifying 17 gene-disease relationships as Definitive (74%), 3 as Moderate (13%), and 3 as Limited (13%) (Figure 2, Supplemental Table 1).

The summaries of all of these curations are stamped with the Cranio GCEP approval date and are publicly available on the ClinGen website (https://search.clinicalgenome.org/kb/gene-validity?page=1&size=25&search=). Nineteen (83%) of the gene-disease relationships are autosomal dominant, 3 (13%) are autosomal recessive, and 1 (4%) is X-linked.

Definitive gene-disease relationships

The Cranio GCEP curated a total of 17 Definitive gene-disease relationships (Supplemental Table 1) for the following genes: *EFNB1, ERF, FGFR1, FGFR2, FGFR3, MSX2, POR, RAB23, SKI, TCF12,* and *TWIST1*. The curated gene-disease relationships had points ranging from 11.2 to 18 points using the ClinGen framework. Definitive curations also require replication over time. At this time, the Cranio GCEP has not curated any Strong gene-disease relationships, as all curations that met the 12-point requirement had evidence of replication over time.

The Cranio GCEP Definitive curations include many of the well-known and widely accepted gene-disease relationships such as *FGFR1*- and *FGFR2*-related Pfeiffer syndrome, *FGFR2*-related Crouzon syndrome and Apert syndrome, and *TWIST1*-related Saethre-Chotzen syndrome(OMIM:101400).

An example of a Definitive gene-disease relationship that required special consideration was Muenke syndrome. Muenke syndrome, which is characterized by uni- or bicoronal synostosis, macrocephaly, midfacial hypoplasia, hearing loss, variable developmental delay and other features, is caused by a specific missense variant in *FGFR3* NM_000142.5:c.749C>G p.(Pro250Arg).[9] Most gene curations focus on highlighting the variant spectrum of a gene-disease assertion. However, since Muenke syndrome is not caused by multiple variants, the Cranio GCEP curated the single recurrent causative variant multiple times in unrelated families. With the help of external expert input, the *FGFR3*-Muenke syndrome curation highlighted the phenotypic variability seen in cases harboring the p.(Pro250Arg) variant, as detailed in the evidence summary.

Moderate gene-disease relationships

The Cranio GCEP curated a total of three Moderate gene-disease relationships (Supplemental Table 1) for the genes *FGFR1, MEGF8,* and *TWIST1*. Total scores ranged from 8.3 to 11 points. This classification indicates promising evidence of the gene-disease relationship, though the evidence from the current literature is ultimately insufficient to reach a Strong or Definitive classification and publication of additional cases is recommended.

Moderate curations in the Cranio GCEP included *FGFR1*-related Hartsfield-Bixler-Demyer syndrome, *MEGF8*-related Carpenter syndrome (OMIM: 614976), and *TWIST1*-related craniosynostosis (OMIM:123100). For example, *TWIST1*-related craniosynostosis reached a Moderate classification with 3.8 points of genetic evidence and 5.5 points for functional evidence. Missense variants and one nonsense variant in *TWIST1* were reported in 8 probands with isolated craniosynostosis in the absence of other syndromic features (Supplemental Table 1). Experimental evidence in support of this gene-disease relationship included expression data and mouse models (Supplemental Table 1). Of note, *TWIST1* had two additional disease assertions which reached Definitive and Limited classifications, respectively (Table 1).

Limited gene-disease relationships

Three rare but clinically defined and distinguishable gene-disease pairs were found to have Limited evidence ranging from 3 to 4 points to support the relationship by the Cranio GCEP: *FGFR1*-related osteoglophonic dysplasia (OMIM:166250), *TWIST1*-related Sweeney-Cox syndrome (OMIM:617746), and *FGFR2* and lacrimo-auriculo-dento-digital (LADD) syndrome (OMIM:149730) (Supplemental Table 1). For example, *FGFR1*-related osteoglophonic dysplasia was first described in 2005, with 5 missense variants, one of which had functional data supporting the variant's impact, reported in 8 probands included in the curation (Supplemental Table 1). The mechanism of disease is suggested to be gain of function variants, causing increased signaling of the FGFR1 protein.[19] There was no experimental evidence available, beyond the variant-level functional data. While no convincing contradictory evidence has emerged, only a Limited classification could be reached due to limited genetic and experimental evidence. Publications of additional probands could help to reach a stronger classification in the future.

Recuration

Moderate and Limited curations will be revisited every three years after their initial approval date to re-assess the evidence from the current literature, as per ClinGen's gene-disease recuration policies.

Discussion

The Cranio GCEP adapted the ClinGen framework to curate genes related to craniofacial malformations, addressing unique challenges in this domain. These

challenges included a limited number of published cases for rare but clinically defined and distinguishable conditions, the predominance of single recurrent hotspot variants (in unrelated individuals) defining specific disease assertions, and an exceptionally broad spectrum of clinical presentations with some conditions having both incomplete penetrance and variable expressivity. Expert discussion and collaboration were essential in navigating these complexities and ensuring a rigorous and consistent application of the ClinGen framework to the extensive craniosynostosis literature. As a result, we curated 23 gene-disease relationships for 12 genes, of which 17 were classified as Definitive, 3 as Moderate, and 3 as Limited.

A critical outcome of this curation process is the categorization of gene-disease relationships based on their strength of evidence. The Cranio GCEP seeks to clarify the clinical relevance of genes involved in craniofacial development and to support laboratory efforts to generate clear, evidence-based results that inform genetic counseling and medical management. In the clinical evaluation of patients with craniosynostosis, distinguishing between syndromic and nonsyndromic forms is critical, and the integration of molecular diagnostic testing into clinical practice has greatly enhanced this differentiation.[24] By delineating the genotypic and phenotypic spectra associated with specific molecular diagnoses, our findings enhance clinicians' ability to perform targeted diagnostic assessments and to guide individualized medical and surgical management for patients with craniofacial malformations. Future studies are needed to better define clinical outcomes in relation to the timing and type of interventions (e.g. clinical monitoring, frontoorbital advancement, calvarial vault remodeling, open vs endoscopic techniques, and postoperative cranial remodeling strategies).

According to ACMG technical standards, genes classified as Moderate, Strong, or Definitive are eligible for inclusion in diagnostic testing panels,[25] whereas those below this threshold should not be considered. This distinction presents a challenge in craniosynostosis; while experts may be aware of internal cases, there are few published cases in the literature. The curation process often required direct engagement with authors of foundational studies to clarify cases. There is an ongoing need for additional published cases to strengthen the evidence-base in this field. Additionally, clinical laboratories should consider submitting variants in craniosynostosis genes to ClinVar with accompanying phenotype data, as these cases can also be scored in curations.[26]

One example of an area where publication of additional cases would be informative is instances of biallelic *FGFR1* variants among individuals with Hartsfield-Bixler-Demyer syndrome. At present, the Cranio GCEP has curated the relationship between *FGFR1* and autosomal dominant Hartsfield-Bixler-Demyer syndrome as moderate, with

functional evidence supporting a dominant negative disease mechanism (e.g., through disruption of RAS/ERK signaling).[27,28] While not included in the current curation, several cases with features of Hartsfield-Bixler-Demyer syndrome, including holoprosencephaly, corpus callosum agenesis, oligodactyly, ectrodactyly, and cleft palate, have been found to harbor homozygous *FGFR1* missense variants, while their heterozygous relatives were unaffected.[29,30] Detailed functional characterization of these specific variants remains lacking. Functional studies by Villanueva et al. 2015 demonstrated a hypomorphic loss-of-function effect for one *FGFR1* missense variant NM_023110.2:c.1286T>A p.(Val429Glu), identified in the homozygous state in a case with ectrodactyly, congenital hypogonadotropic hypogonadism, and anosmia.[31] *FGFR1* variants have been linked to skeletal anomalies such as hemivertebrae and limb malformations in autosomal dominant Kallmann syndrome, which is caused by haploinsufficiency. Such skeletal malformations are phenocopied in FGFR1-deficient mouse models.[30] *FGFR1* is a pleiotropic developmental regulator. It is possible that in reducing its dose below 0.5, either through a monoallelic dominant negative variant, or biallelic hypomorphic loss of function variants, additional organ systems such as midline brain and limb development become increasingly affected. However, direct functional evidence for the biallelic variants described in cases with features of Hartsfield-Bixler-Demyer syndrome is needed to support this. This gene highlights some of the complexities encountered in curation and underscores the need for publication of additional cases and functional studies. In the future, as more cases are published, we plan on recurating the relationship between biallelic *FGFR1* variants and Hartsfield-Bixler-Denmyer syndrome.

Lumping and splitting genetic skeletal disorders, including those with craniofacial manifestations described in this current work, has been a long-standing area of inquiry. The original nosology of skeletal disorders was published in 1971[32] and has subsequently undergone 11 revisions, with the most recent published in 2023.[14] The nosology has characterized disorders based on radiographic criteria, biochemical criteria, and, more recently, functional and molecular criteria.[14] The nosology may split disease entities based on clinical presentation and severity, even when the underlying molecular mechanism is the same, as this has utility for clinical management.[14] In contrast, the ClinGen's framework for lumping and splitting heavily weights the underlying molecular mechanism in lumping/splitting determinations, while also considering the phenotypic spectrum and mode of inheritance.[13] This is aligned with ClinGen's mission to curate gene-disease relationships and their evidence for use in clinical molecular genetic testing, which is distinct from the nosology's goals. Clinical distinctions delineated in the nosology may therefore not match with the distinct molecular mechanisms required to separate disease entities in the ClinGen framework. In cases where disease entities were lumped based on the ClinGen framework, the

corresponding evidence summary described the entities included within the lumped assertion.

Additionally, the Cranio GCEP carefully considered the evolving landscape of craniofacial malformations literature, incorporating both historical perspectives and contemporary classification systems to provide a comprehensive and robust curation. The variability in disease nomenclature—ranging from gene-based names to eponyms and phenotype-driven classifications—further complicated this process. In alignment with ClinGen's Disease Naming Guidelines, the Cranio GCEP supports dyadic naming conventions while recognizing the continued use of historical terminology.[33] Notably, the 2023 revision of the nosology of skeletal disorders also supports the dyadic naming approach in which the main phenotypic descriptor is coupled with the causative gene.[14] This not only facilitates clarity in clinical and laboratory settings but also establishes a consensus framework that will guide future research and publications.

To ensure continued refinement of craniofacial gene-disease curation, the Cranio GCEP encourages the publication of cases involving previously identified genes, recognizing the barriers to publishing reports on ultra-rare diseases. When formal publication is not feasible, we advocate for the submission of variants to resources such as NCBI's ClinVar or Matchmaker Exchange to maintain the integrity of the evidence-base.[34,35] These efforts will contribute to ClinGen's recuration process, with Round 1 genes scheduled for recuration beginning in 2025.

Future work

The Cranio GCEP is currently curating a round two gene list, focusing on those in which pathogenic variants have been reported in mandibulofacial dysostosis, cleft lip and/or palate, and additional causes of craniosynostosis: *ALX1, ALX3, ALX4, ARHGAP29, CTNND1, DHODH, EFTUD2, FOXE1, GRHL3, IL11RA, IRF6, KMT2D, MSX1, NECTIN1, POLR1B, POLR1C, POLR1D, SATB2, SF3B4, SMAD6, SMO, TBX22, TGDS, TP63, ZIC1, ZSWIM6.*

Data Availability

Curations from the Craniofacial Malformations GCEP are publicly available on the Clinical Genome Resource website (https://search.clinicalgenome.org/kb/gene-validity/). The publication's data is also available in the Supplemental Materials.

Acknowledgements

The Cranio GCEP thanks Dr. Maximillian Muenke, Dr. Amy Merrill, and all other expert contributors for their participation as expert guests in ClinGen curation and for clarifying case presentations.

Funding Statement

This publication was supported by the National Human Genome Research Institute of the National Institutes of Health through the following grants: U24HG006834. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Chloe Mighton received support from the Canadian Institutes of Health Research (CIHR, FRN #510986).

Author Contributions

Conceptualization: E.E., C.M., P.A.S., E.W.J., A.G., M.H., H.M., M.P., E.H.W., M.M, M.D.; Formal Analysis, Visualization: E.E., C.M.; Investigation: E.E., C.M., S.G., A.G., M.H., H.M., M.P., E.H.W., M.M.; Writing-original draft: E.E., C.M.; Writing-review and editing: E.E., C.M., E.B., V.N.G., J.R., M.D., S.G., A.G., M.H., H.M., M.P., E.H.W., M.M., C.P., H.M.S., E.S., A.O.M.W., E.Z., Y.A.Z., J.M.G., E.W.J., P.A.S.

Ethics Declaration

The research outlined in this study does not involve human participants or animal models.

Conflict of Interest

The authors declare no relevant conflicts of interest.

Additional Information

The online version of this article contains supplementary material, which is available to authorized users.

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Figure 1: The Craniofacial Malformations Gene Curation Expert Panel's modifications to the ClinGen scoring matrix for Genetic Evidence, with increased points awarded for cases with radiographic evidence showing the variant's

phenotypic impact. The remaining scores are consistent with the standard ClinGen scoring framework.

Figure 2. Total amount of points awarded for each gene-disease relationship. The lines indicate the number of points required to reach a moderate (7 points, blue line) and strong/definitive (12 points, red line) gene-disease relationship.

Table 1: Craniofacial Malformations Round 1 Precuration Decision.

Gene (HGNC ID)	MONDO Disease Assertion (OMIM ID)	MOI	Phenotypes	Molecular Mechanism	Lump/Split	Decision
EFNB1 (HGNC:3226)	craniofrontonasal syndrome (OMIM: 304110)	XL	Females have frontonasal dysplasia, craniofacial asymmetry, craniosynostosis, bifid nasal tip, grooved nails, wiry hair, and abnormalities of the thoracic skeleton, whereas males typically show only hypertelorism	Loss of function (cellular interference) [36]		Curated single assertion
ERF (HGNC:3444)	Chitayat syndrome (OMIM:617180)	AD	Respiratory distress presenting at birth, bilateral accessory phalanx resulting in shortened index fingers with ulnar deviation, hallux valgus, and characteristic facial features including prominent eyes, hypertelorism, depressed nasal bridge, full lips, and upturned nose	Unknown. At time of precuration, all reported cases had recurrent NM_006494.4: c.266A>G p.(Tyr89Cys) variant	Split	Curated ERF for craniosynostosis 4 (MONDO:0010929)
	craniosynostosis 4 (OMIM:600775)	AD	Isolated craniosynostosis	Haplo-insufficiency		
FGFR1 (HGNC:3688)	osteoglophonic dysplasia[a] (OMIM:166250)	AD	Rhizomelic dwarfism, nonossifying bone lesions, craniosynostosis, prominent supraorbital ridge, and depressed nasal bridge	Constitutive gain of Function	Split	Curated each assertion separately. Lump Jackson-Weiss syndrome and Pfeiffer syndrome and curated for Pfeiffer syndrome 1. OMIM contains an assertion for Jackson-Weiss syndrome syndrome and *FGFR1* (123150), which notes that only a single case

	Hartsfield-Bixler-Demyer syndrome (OMIM:615465)	AD	Holoprosencephaly, ectrodactyly, and cleft/lip palate. Intellectual disability, and multiple other congenital anomalies usually occur. The disorder involves midline and limb field defects	Dominant negative		was described.[37]Given the phenotypic overlap, same causative variant NM_023110.3:c.755C>G (p.Pro252Arg), and same inheritance pattern in this case as in *FGFR1*-related Pfeiffer syndrome, Jackson-Weiss syndrome was lumped into Pfeiffer syndrome. Note, Jackson-Weiss syndrome is caused by *FGFR2* variants.
	Jackson-Weiss syndrome (OMIM:123150)	AD	Craniosynostosis characterized by premature fusion of the cranial sutures as well as radiographic anomalies of the feet	Gain of Function	Lump into Pfeiffer syndrome type 1 (MONDO: 0019659)	
	FGFR1-related Pfeiffer syndrome[a] (OMIM:101600)	AD	Craniosynostosis syndrome with characteristic anomalies of the hands and feet.	Ligand-dependent gain of Function		
FGFR2 (HGNC:3689)	Apert syndrome (OMIM:101200)	AD	Craniofacial anomalies and syndactyly of the hands and feet	Ligand-dependent gain of Function	Split	Curated each assertion separately
	Crouzon syndrome (OMIM:123500)	AD	Craniosynostosis and facial abnormalities with a lack of major hand and feet abnormalities	Constitutive gain of Function		
	FGFR2-related Pfeiffer syndrome[a] (OMIM:101600)	AD	Characterized by broad and medially deviated thumbs and great toes, proptosis, syndactyly in some cases, and cloverleaf skull in more severe cases	Gain of Function		
	Beare-Stevenson cutis gyrata syndrome (OMIM:123790)	AD	Cloverleaf skull deformity and deep skin folds and on the scalp, palms, soles, and other areas of the body	Constitutive gain of Function		

	LADD syndrome (lacrimo-auriculo-dento-digital) (OMIM:149730)	AD	A multiple congenital anomaly disorder mainly affecting lacrimal glands and ducts, salivary glands and ducts, ears, teeth, and distal limb segments	Dominant negative		
	FGFR2-related bent bone dysplasia (OMIM:614592)	AD	Poor mineralization of the calvarium, craniosynostosis, dysmorphic facial features, prenatal teeth, hypoplastic pubis and clavicles, osteopenia, and bent long bones	Gain of Function		
FGFR3 (HGNC:3690)	Crouzon syndrome-acanthosis nigricans syndrome (OMIM:612247)	AD	Crouzon syndrome-acanthosis nigricans syndrome	Gain of Function	Split	*FGFR3* was curated by the Skeletal GCEP for achondroplasia, SADDAN, hypochondroplasia, *FGFR3*-related thanatophoric dysplasia (curation pending)(OMIM:187601) and CATSHL syndrome(OMIM:610474). *FGFR3* was curated by the Cranio GCEP for Crouzon and Muenke syndromes.
	Muenke syndrome (OMIM:602849)	AD	Uni- or bicoronal synostosis, macrocephaly, midfacial hypoplasia, and developmental delay. Other more variable features include thimble-shaped middle phalanges, brachydactyly, carpal/tarsal fusion, and deafness.	Ligand-dependent gain of Function		
	Achondroplasia (OMIM:100800)	AD	Short stature caused by rhizomelic shortening of the limbs, characteristic facies with frontal bossing and midface hypoplasia, exaggerated lumbar lordosis, limitation of elbow extension,	Gain of Function		

			genu varum, and trident hand			
	SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) (OMIM: 616482)	AD	Ram's horn shaped clavicles and reverse bowing of lower limbs.	Gain of Function		
	thanatophoric dysplasia type I (OMIM:187600)	AD	Short femurs with or without cloverleaf skull were designated TD type I (TD1)	Gain of Function		
	thanatophoric dysplasia type II (OMIM:187601)	AD	Patients with straight, relatively long femurs always had associated severe cloverleaf skull	Gain of Function		
	CATSHL syndrome (camptodactyly-tall stature-hearing loss) (OMIM:610474)	SD	Camptodactyly, tall stature, and hearing loss syndrome	Dominant negative		
MEGF8 (HGNC:3233)	*MEGF8*-related Carpenter syndrome (OMIM:614976)	AR	Multiple congenital malformation disorder characterized by multisuture craniosynostosis and polysyndactyly of the hands and feet, in association with abnormal left-right patterning and other features, most commonly obesity, umbilical hernia, cryptorchidism, and congenital heart disease	Loss of Function		Curated single assertion
MSX2 (HGNC:7392)	craniosynostosis 2 (OMIM:604757)	AD	Isolated craniosynostosis	Gain of Function	Split	Curated each assertion separately
	parietal foramina (OMIM:168500)	AD	Isolated parietal foramina	Haplo-insufficiency		

POR (HGNC:9208)	Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis (OMIM:201750)	AR	Midface hypoplasia, radiohumeral synostosis, femoral bowing, and joint contractures. Phenotypes specific to *POR*-related Antley-Bixler include steroidogenesis and genital anomalies.	Loss of Function	Lump genital anomaly and steroidogenesis phenotypes into one assertion	Curated single assertion
RAB23 (HGNC:14263)	*RAB23*-related Carpenter syndrome (OMIM:201000)	AR	Cardinal features of acrocephaly with variable synostosis of the sagittal, lambdoid, and coronal sutures; peculiar facies; brachydactyly of the hands with syndactyly; preaxial polydactyly and syndactyly of the feet; congenital heart defects; growth retardation; intellectual disabilities; hypogenitalism; and obesity. In addition, cerebral malformations, oral and dental abnormalities, coxa valga, genu valgum, hydronephrosis, precocious puberty, and hearing loss may be observed	Loss of Function		Curated single assertion

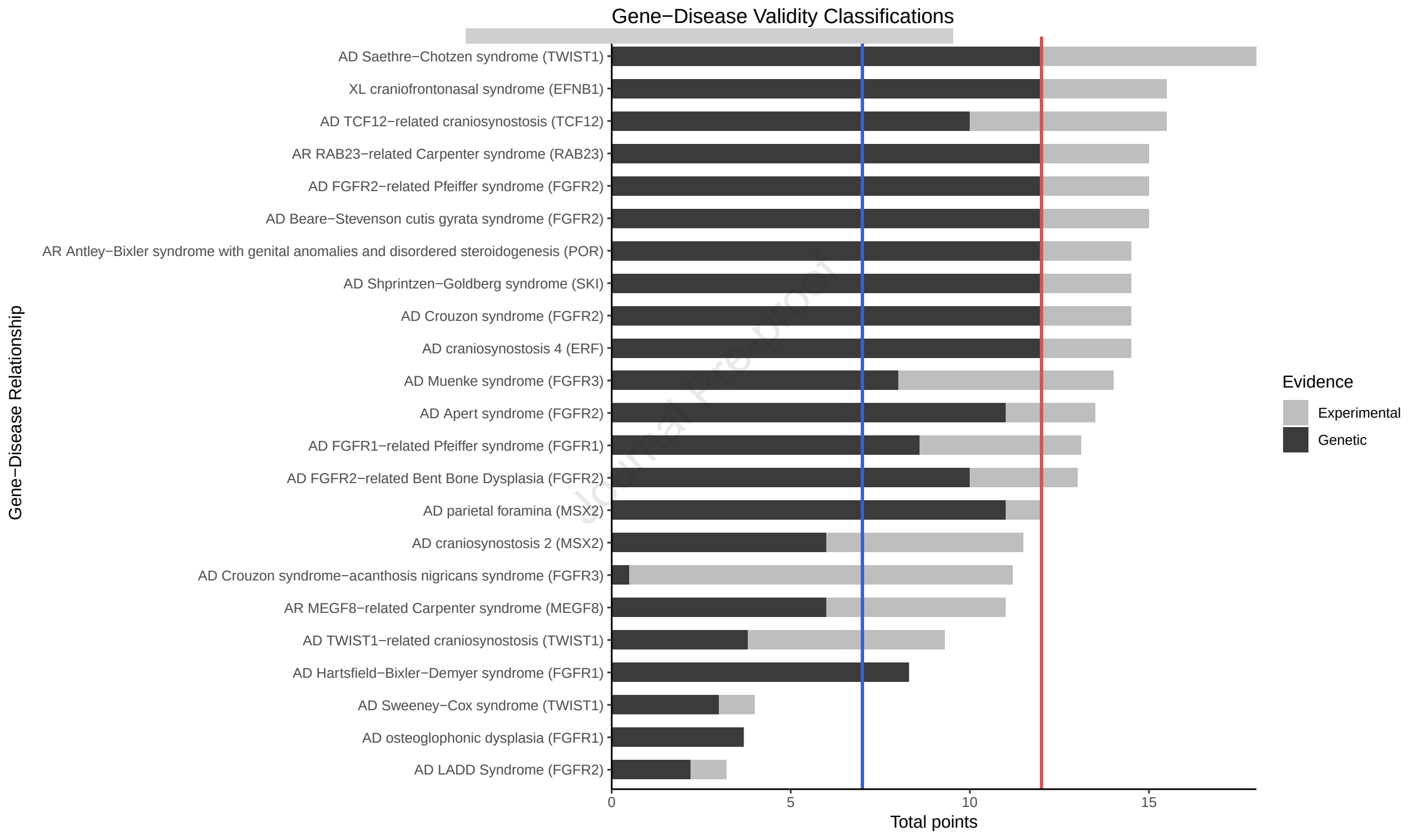
SKI (HGNC:10896)	Shprintzen-Goldberg syndrome (OMIM: 182212)	AD	Craniosynostosis and craniofacial features, motor and cognitive delays, mild-moderate intellectual disability, hypotonia, musculoskeletal anomalies, and cardiovascular problems such as aortic aneurysm	Gain of Function		Curated single assertion
TCF12 (HGNC:11623)	*TCF12*-related craniosynostosis (OMIM:615314)	AD	Fusion of coronal sutures and individuals can present with other syndromic features including dental malocclusion, blepharoptosis, dysmorphic appearance of the face and external ears, and minor limb anomalies	Haplo-insufficiency		Curated single assertion
TWIST1 (HGNC:12428)	Saethre-Chotzen syndrome (OMIM:101400)	AD	Bicoronal synostosis causing brachycephaly or acrocephaly, high forehead with low anterior hairline, ptosis, hypertelorism, lacrimal duct stenosis, high palate, small ears with prominent crus, facial asymmetry, partial cutaneous syndactyly of hands and feet, and hallux valgus	Haplo-insufficiency	Split	Curate each assertion separately
	Sweeney-Cox syndrome (OMIM:617746)	AD	Distinct facial dysostosis. Features include hypertelorism, deficiencies of the eyelids and facial bones, cleft palate/velopharyngeal insufficiency, and low-set cupped ears	Dominant negative		

	TWIST1-related craniosynostosis (OMIM:123100)	AD	Isolated craniosynostosis	Loss of Function		

MOI, mode of inheritance; AD, autosomal dominant inheritance; AR, autosomal recessive, XL, X-linked

[a]A MONDO request has been put in for the following curations: *FGFR1*-related Pfeiffer syndrome, *FGFR2*-related Pfeiffer syndrome, and *FGFR1*-related osteoglophonic dysplasia. A name update will be made but the genes have currently been curated for Pfeiffer syndrome type 1 (MONDO:0019659), Pfeiffer syndrome (MONDO:0007043), and osteoglophonic dwarfism (MONDO:0008150).

Variant Type (Starting Score)	Suggested Upgrades		
	Functional Data	***De Novo***	**Radiographic evidence**
Predicted or proven null (1.5 points)	+0.5 points	+0.5 points	+0.5 points
Other variant type (0.1 points)	+0.4 points	+0.4 points	+0.4 points


Gene−Disease Validity Classifications
AD Saethre−Chotzen syndrome (TWIST1)
XL craniofrontonasal syndrome (EFNB1)
AD TCF12−related craniosynostosis (TCF12)
AR RAB23−related Carpenter syndrome (RAB23)
AD FGFR2−related Pfeiffer syndrome (FGFR2)
AD Beare−Stevenson cutis gyrata syndrome (FGFR2)
AR Antley−Bixler syndrome with genital anomalies and disordered steroidogenesis (POR)
AD Shprintzen−Goldberg syndrome (SKI)
AD Crouzon syndrome (FGFR2)
AD craniosynostosis 4 (ERF)
AD Muenke syndrome (FGFR3)
AD Apert syndrome (FGFR2)
AD FGFR1−related Pfeiffer syndrome (FGFR1)
AD FGFR2−related Bent Bone Dysplasia (FGFR2)
AD parietal foramina (MSX2)
AD craniosynostosis 2 (MSX2)
AD Crouzon syndrome−acanthosis nigricans syndrome (FGFR3)
AR MEGF8−related Carpenter syndrome (MEGF8)
AD TWIST1−related craniosynostosis (TWIST1)
AD Hartsfield−Bixler−Demyer syndrome (FGFR1)
AD Sweeney−Cox syndrome (TWIST1)
AD osteoglophonic dysplasia (FGFR1)
AD LADD Syndrome (FGFR2)
Gene−Disease Relationship
0
5
10
15
Total points
Evidence
Experimental
Genetic
Journal Pre-proof