symmetry

MDPI

Systematic Review

Genetic Factors That Affect Asymmetric Mandibular Growth—A Systematic Review

Alicja Babczyńska [1,*,†], Beata Kawala [1,2,†] and Michał Sarul [3,4,†]

[1] Clinic of Orthodontics, Academic Policlinic of Stomatology, 50-425 Wrocław, Poland; beata.kawala@umw.edu.pl
[2] Department of Orthodontics and Dentofacial Orthopedics, Wroclaw Medical University, 50-425 Wrocław, Poland
[3] Department of Integrated Dentistry, Wroclaw Medical University, 50-425 Wrocław, Poland; michal.sarul@umw.edu.pl
[4] Cilinic of Integrated Dentistry, Academic Policlinic of Stomatology, 50-425 Wrocław, Poland
* Correspondence: ortodoncja@umw.edu.pl
† Current address: 1, 2, 3, 4, Krakowska 26, 50-425 Wrocław, Poland.

Abstract: Facial asymmetry is a feature that occurs to a greater or lesser extent in the general population. As its severity is usually slight, facial asymmetry may not be noticeable to the patient. However, there are cases when severe facial asymmetry not only affects the facial aesthetics by distorting facial proportions, but also contributes to problems related to the function of the stomatognathic system. The nodal signalling pathway appears to be of particular importance in the process of mandibular asymmetry, as it affects not only structures formed from the first pharyngeal arch, but also other organs, such as the heart and lungs. Following the evaluation of the available literature, the inheritance of mandibular asymmetry is a very complex and multifactorial process, and the genes whose altered expression appears to be a more important potential aetiological factor for asymmetry include PITX2, ACTN3, ENPP1 and ESR1. This systematic review attempts to systematise the available literature concerning the impact of signalling pathway disruption, including the disruption of the nodal signalling pathway, on the development of mandibular asymmetry.

Keywords: mandibular asymmetry; systematic review; nodal pathway; PITX2; ACTN3; ENPP1; ESR1

Citation: Babczyńska, A.; Kawala, B.; Sarul, M. Genetic Factors That Affect Asymmetric Mandibular Growth—A Systematic Review. *Symmetry* **2022**, *14*, 490. https://doi.org/10.3390/sym14030490

Academic Editors: Anna Paradowska-Stolarz, Irena Duś-Ilnicka and Maria Cristina Pollmann

Received: 29 January 2022
Accepted: 25 February 2022
Published: 28 February 2022

1. Introduction

Facial asymmetry is a feature that occurs to a greater or lesser extent in the general population. According to a systematic review by Evangelista et al. [1], who examined the prevalence of mandibular asymmetry in skeletal sagittal patterns, mandibular asymmetry ranged from 17.43 percent to 72.95 percent in overall samples. To plan the treatment of a patient with facial asymmetry in a proper way, a detailed diagnosis must be performed, taking into account the fact that not all abnormalities are pathological and require treatment [2]. As its severity is usually slight, the asymmetry may not be noticeable to the patient. However, there are cases when severe facial asymmetry not only affects the facial aesthetics by distorting facial proportions, as the perception of symmetry between the two sides of the face defines attractiveness [3], but also contributes to problems related to the function of the stomatognathic system. In Obwegeser's opinion [4], the causes of asymmetry can be divided into three groups according to the time of onset: those occurring in the process of embryogenesis, those occurring postnatally and those resulting from faulty growth regulation of unknown aetiology. The clinical manifestation of group 1 defects can be varied. Asymmetry can be caused by either unilateral hypoplasia or aplasia of the condylar process, unilateral hypoplasia or aplasia of the mandible, unilateral hypoplasia of the mandible and face, or hyperplasia of the entire half of the face, or underdevelopment of half of the face. As a consequence of the underdevelopment of the condylar process and

the reduced height of the rami mandibulae, several significant abnormalities may occur, e.g., in the course of the occlusal plane, and cause several types of defects, the treatment of which can be a challenge for orthodontists and maxillofacial surgeons.

To understand the role of genetic factors in the development of mandibular asymmetry, it is necessary to identify embryogenesis [5], its stages and the factors that affect growth. The tissues of the craniofacial complex are mainly derived from neural crest cells (NCCs), a population of temporarily migrating cells that arise from the dorsal part of the neural tube during embryogenesis and then they migrate to populate the frontonasal process (FNP), first, second, third and fourth pharyngeal arches [6]. NCCs contribute to the development of many cell and tissue types throughout the body (enteric nervous system, glia, neurons, melanocytes, connective tissue, chondrocytes and myofibroblasts lining the blood vessels) [6]. Understanding the importance and complexity of this process seems to be essential to understand the aetiopathogenesis of defects that affect craniofacial structures. The presence of aberrations in any of the multistep processes involved in the regulation of NCC behaviour can result in developmental defects. Several signalling pathways were found to be necessary for NCC generation and/or survival, including two bone morphogenetic protein (BMP) antagonists, chordin and noggin [7], fibroblast growth factors (FGFs) [8], Wnt signals [9] and nodal signalling pathway.

The latter plays a fundamental role in the formation of the mesoderm and endoderm, the modelling of the anterior neural plate and the identification of bilateral asymmetry in vertebrates. Its asymmetric expression induces the expression of PITX2, a gene that belongs to homeobox genes involved in, among other things, the regulation of organogenesis in all eukaryotes. Apart from the PITX2 mutation, variants of the ACTN3, PITX1, ENPP1, and ESR1 genes are also most often associated with asymmetry within the mandible [10–13].

The correct diagnosis, treatment and prevention of mandibular asymmetry requires a thorough understanding of the pathogenetic mechanisms that lead to it. Determining the role of mutations in individual genes may contribute to a better understanding of the mechanisms of mandibular growth and its disorders. It would also allow for a more complete diagnosis of patients during the qualification for functional/surgical treatment. It is also crucial in the genetic counselling of patients with abnormal facial morphology [11,13].

This systematic review focuses on the genetic factors associated with the development of mandibular asymmetry during embryogenesis.

2. Methods

This study mainly aims to try to answer the question "What genetic factors affect asymmetric mandibular growth?" The following systematic review was written based on the principles detailed in Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

3. Information Sources

The primary sources of information regarding the factors that affect asymmetric mandibular growth were four databases: PubMed, Ovid Medline, Web of Science and Cochrane Reviews. All the data were retrieved from articles published until 30 November 2021.

4. Search Strategy

Different keywords were used to finally select the information and research that best answer the question "What genetic factors affect asymmetric mandibular growth?" Due to the nature of the issue under investigation and to clarify and systematise the available knowledge thoroughly, both human and animal studies were included in this analysis. The search was conducted in the following databases: PubMed, Ovid Medline, Web of Science and Cochrane Library. The following combinations of keywords were used: mandibular asymmetry, genetic, hereditary, congenital, facial asymmetry. The selection of articles was initiated by removing internal and external duplicates (3696 duplicates were found). When analysing the results obtained, it was also checked whether there was already

an article covering the topic of this systematic review. No similar publications were found. Then, after the inclusion and exclusion criteria were established for abstracts, all titles and abstracts were analysed bearing in mind the previously posed question "What genetic factors affect asymmetric mandibular growth?" The date of publication was a primary search criterion. All selected articles had to be published in the following period: 1 January 1991–30 November 2021.The specified date range was established after the initial database screening, taking into account the publication dates of the articles. Another criterion that the articles had to meet to be included in this systematic review was whether they fell into the following categories: Clinical Trials and Randomised Controlled Trials. The following discarded articles included reviews, systematic reviews, case reports and articles that describe treatments or diagnostic methods of mandibular and facial asymmetry, as well as articles that do not directly concern the mandible (Table 1 and Figure 1).

Table 1. The summary of findings retrieved from a search of articles concerning genetic factors that affect asymmetric mandibular growth.

Database	The Date the Search Was Performed	Number of All Articles Searched	Number of All Articles	Number of Internal and External Duplicates	Number of All Articles after the Removal of Duplicates
PubMed	25 November 2021–20 December 2021	7843			
Ovid Medline	25 November 2021–20 December 2021	79	8258	3696	4562
Cochrane	25 November 2021–20 December 2021	16			
Web of Science	25 November 2021–20 December 2021	320			

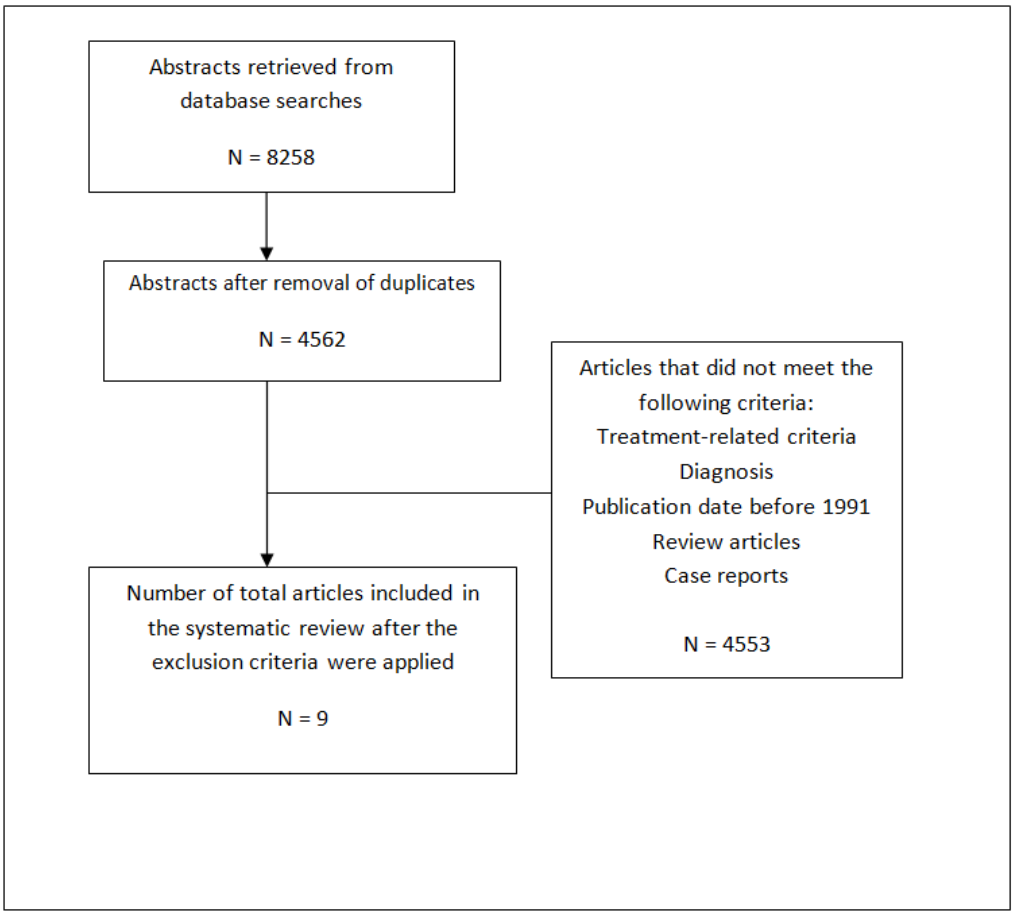

Figure 1. Flow chart.

5. Qualitative Assessment-Risk of Bias

The risk of bias (RoB) assessment tool, adapted according to the type of study, was used for verifying the likelihood of a confounding result. To analyse the quality of research, the found articles were divided into two groups, namely, those including human cases and those including animal cases. To assess the quality of human studies, the JBI critical appraisal tool was used separately for studies with a control group (JBI critical appraisal tool: checklist for analytical case–control studies) and without a control group (JBI critical appraisal tool: checklist for cross-sectional studies) [14]. The choice of these tools was dictated by the recommendations included in the review by Lin-Lu et al. [15].

To assess the animal study by Shi et al., (2021) [16], we used the JBI critical appraisal tool: checklist for analytical case–control studies, as this was the only non-experimental study that was conducted in this group. To analyse the remaining animal studies, the SYRCLE's risk of bias (RoB) tool for animal studies (the Systematic Review Centre for Laboratory Animal Experimentation) was used, which was developed from the Cochrane RoB Tool [17] and adapted to account for differences between aspects of animal and human studies.

6. Results

After the first selection, there were five human studies that met the above-mentioned requirements: two cross-sectional studies [13,18] and three case–control studies [11,19,20]. Four animal studies were also selected: one case–control study [16] and three experimental studies [21–23].

Risk of Bias Assessment

Table 2 shows the results obtained in our analysis based on the JBI critical appraisal tool: checklist for case–control studies. According to our assessment, the article by Nicot et al. (2014) had the lowest RoB, whereas in the other two publications, the risk was only slightly higher. A certain problem in the Nicot et al. (2014) study was the exclusion of one patient with symmetry from the control group, as the principal component analysis (PCA) revealed that the patient had the largest difference in terms of global gene expression compared to the ten remaining patients. Both this and the lack of quantitative analysis of facial asymmetry seem to imply that the aforementioned study has a higher RoB than originally assessed.

Unfortunately, due to the specificity of the topic under study, not all the questions included in the RoB assessment tool were applied to our analysis. In the case of genetic testing, the exposure period does not appear to affect the research result, so the posed question does not seem to be adequate. The identification of confounding factors and methods of how to deal with them also proved to be problematic. Then, the RoB assessment was conducted for human cross-sectional studies according to the JBI critical appraisal tool: checklist for cross-sectional studies (Table 3). There were similar problems as in the previous analysis. The analysed human cross-sectional studies appear to have a higher RoB compared to the previously discussed clinical case studies. According to our assessment, the main additional problem was the lack of appropriate statistical analysis for the results obtained in the study by Sofyanti et al., (2018) [18]. For this reason, the entire study has a higher RoB compared to the second human cross-sectional study by Nicot et al., (2020).

Another important aspect in the assessment of RoB, not flagged in the JBI critical appraisal tool analysis, is how to assess asymmetry in human studies. All authors used two-dimensional radiographs for this purpose. Authors used different types of radiographs: Sofyanti et al. [18,20] analysed asymmetry with panoramic radiograph and Chung et al. [11] and Nicot et al. [13] used cephalometric analysis. In article [19], Nicot et al. performed diagnosis using different types of radiographs. Unfortunately, these images are not as accurate as, for example, CBCT images that enable more precise measurements and using different types of radiographs makes studies difficult to compare.

Then, animal studies were analysed. As that was the only non-experimental study, a case–control study by Shi et al., (2021) was selected and analysed according to the JBI's critical appraisal tool: checklist for case–control studies (Table 4). The analysis revealed that

the above-mentioned study had a higher RoB compared to the same type of human study. The analysis of other animal studies according to the SYRCLE's RoB tool (Table 5) further confirmed the aforementioned thesis. Most of the obtained responses were "High risk of bias" or "Unclear", which indicates a relatively high risk of misleading conclusions. Then, the GRADE (Grading of Recommendations, Assessment, Development and Evaluations) analysis was conducted for the findings obtained (Figures 2 and 3).

Admission type	Study design	Risk of Bias (RoB)	Inconsistency	Indirectness	Imprecision	Comments	Certainty
Certainty assessment							
Title of publication, authors							
"Nodal Pathway Genes Are Down-regulated in Facial Asymmetry" Nicot et al. 2014	Case-control study	Serious	Not serious	Not serious	Very serious	A very small study group, however, the thesis was also confirmed in other publications	⊕⊕OO Low
"ENPP1 and ESR1 genotypes associated with subclassifications of craniofacial asymmetry and severity of temporomandibular disorders" Chung et al. 2017	Case-control study	Serious	Not serious	Not serious	Not serious		⊕⊕⊕O Moderate
"Exclusion of pituitary homeobox 2 gene polymorphism in vertical mandibular asymmetry patients: a preliminary study" Sofyanti et al. 2018 [11]	Case-control study	Serious	Not serious	Not serious	Not serious		⊕⊕⊕O Moderate
"Condyle modelling stability, craniofacial asymmetry and ACTN3 genotypes: Contribution to TMD prevalence in a cohort of dentofacial deformities" Nicot et al. 2020	Cross-sectional study	Serious	Not serious	Not serious	Not serious		⊕⊕⊕O Moderate
"Polymorphism in 4'- UTR Region of PITX2 in Vertical Mandibular Symmetry" Sofyanti et al. 2018 [8]	Cross-sectional study	Serious	Not serious	Not serious	Serious	A small study group underestimates the level of certainty	⊕⊕⊕O Moderate

Figure 2. The GRADE summary of findings (human studies).

Admission type	Study design	Risk of Bias (RoB)	Inconsistency	Indirectness	Imprecision	Comments	Certainty
Certainty assessment							
Title of publication, authors							
"Analysis of DNA Methylation Profiles in Mandibular Condyle of Chicks with Crossed Beaks Using Whole-Genome Bisulfite Sequencing" Shi et al. 2021	Case-control animal study	Serious	Very serious	Serious[a]	Very serious	A small study group and lack of other studies that support the thesis	⊕OOO Very low
"Sulfotransferase Ndst1 is Needed for Mandibular and TMJ Development" Yasuda et al. 2010	Experimental animal study	Very serious	Very serious	Serious[a]	Not serious	No other studies are available to support the thesis	⊕OOO Very low
"PITX2 Deficiency Results in Abnormal Ocular and Craniofacial Development in Zebrafish" Liu et al. 2012	Experimental animal study	Very serious	Not serious	Serious[a]	Not serious		⊕⊕OO Low
"YPEL1 Overexpression in Early Avian Craniofacial Mesenchyme Causes Mandibular Dysmorphogenesis by Up-Regulating Apoptosis" Yang Tan et al. 2015	Experimental animal study	Very serious	Very serious	Serious[a]	Serious	No other studies are available to support the thesis	⊕OOO Very low
[a] – There is no evidence that the effect obtained in the animal study will cause the same effect in humans							

Figure 3. The GRADE summary of findings (animal studies).

Table 2. The analysis of the quality of human studies with a control group according to the JBI critical appraisal tool: checklist for case–control studies.

		Nicot et al., 2014	Chung et al., 2017	Sofyanti et al., 2018 [20]
1	Were the groups comparable beyond the presence of disease in cases or the absence of disease in controls?	Yes	Yes	Yes
2	Were cases and controls matched appropriately?	Yes	Yes	Yes
3	Were the same criteria used for the identification of cases and controls?	Unclear	Yes	Yes
4	Was the exposure measured in a standard, valid and reliable way?	Not applicable	Not applicable	Not applicable
5	Was the exposure measured in the same way for cases and controls?	Not applicable	Not applicable	Not applicable
6	Were confounding factors identified?	Unclear	No	No
7	Were strategies to deal with confounding factors stated?	No	No	No
8	Were outcomes assessed in a standard, valid and reliable way for cases and controls?	Yes	Yes	Yes
9	Was the exposure period of interest long enough to be meaningful?	Not applicable	Not applicable	Not applicable
10	Was an appropriate statistical analysis used?	Yes	Yes	Yes

Possible answers: Yes/No/Unclear/Not applicable.

Table 3. The analysis of the quality of human studies without a control group according to the JBI critical appraisal tool: checklist for cross-sectional studies.

		Nicot et al., 2020	Sofyanti et al., 2018 [18]
1	Were the criteria for inclusion in the sample clearly defined?	Yes	Yes
2	Were the study subjects and the setting described in detail?	No	Yes
3	Was the exposure measured validly and reliably?	Yes	Yes
4	Were objective standard criteria used for measurement of the condition?	Yes	Yes
5	Were confounding factors identified?	No	No
6	Were strategies to deal with confounding factors stated?	No	No
7	Were the outcomes measured validly and reliably?	Yes	Yes
8	Was an appropriate statistical analysis used?	Yes	No

Possible answers: Yes/No/Unclear/Not applicable.

Table 4. The analysis of the quality of animal studies with a control group according to the JBI critical appraisal tool: checklist for case–control studies.

		Shi et al., 2021
1	Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?	Yes
2	Were cases and controls matched appropriately?	Yes
3	Were the same criteria used for the identification of cases and controls?	Unclear
4	Was the exposure measured in a standard, valid and reliable way?	Yes
5	Was the exposure measured in the same way for cases and controls?	Yes
6	Were confounding factors identified?	No
7	Were strategies to deal with confounding factors stated?	Not applicable
8	Were outcomes assessed in a standard, valid and reliable way for cases and controls?	Yes
9	Was the exposure period of interest long enough to be meaningful?	Not applicable
10	Was an appropriate statistical analysis used?	Yes

Possible answers: Yes/No/Unclear/Not applicable.

Table 5. The analysis of the quality of human studies with a control group according to the JBI critical appraisal tool: checklist for case–control studies.

			Yasuda et al., 2010	Liu et al., 2012	Yang Tan et al., 2015
	Type of Bias	**Domain**			
Was the allocation sequence adequately generated and applied?	Selection bias	Sequence generation	High risk of bias	High risk of bias	High risk of bias
Were the groups similar at baseline or were they adjusted for confounders in the analysis?	Selection bias	Baseline characteristics	Unclear	Unclear	Unclear
Was the allocation adequately concealed?	Selection bias	Allocation concealment	High risk of bias	High risk of bias	Unclear
Were the animals randomly housed during the experiment?	Performance bias	Random housing	Low risk of bias	Low risk of bias	Low risk of bias
Were the caregivers and/or investigators blinded from the knowledge of which intervention each animal received during the experiment?	Performance bias	Blinding	Unclear	Unclear	Unclear
Were animals selected at random for outcome assessment?	Detection bias	Random outcome assessment	Unclear	Unclear	Unclear
Was the outcome assessor blinded?	Detection bias	Blinding	Unclear	Unclear	Unclear
Were incomplete outcome data adequately addressed?	Attrition bias	Incomplete outcome data	Unclear	Unclear	High risk of bias
Are reports of the study free of selective outcome reporting?	Reporting bias	Selective outcome reporting	Unclear	Unclear	High risk of bias
Was the study apparently free of other problems that could result in a high risk of bias?	Other	Other sources of bias	High risk of bias	Unclear	Unclear

7. Discussion of Outcomes

The most frequent keywords used during the analysis of selected articles were nodal signalling pathway and the PITX2 gene, which were considered to play a major role in asymmetry formation.

7.1. PITX2

The impact of this gene was analysed in more than half of publications, five out of nine articles. The articles that addressed these issues were human studies, both publications by Sofyanti et al., 2018 [18,20], Nicot et al., 2014 [19], Chung et al., 2017 [11], as well animal studies, Liu et al., 2012 [21]. Cross-sectional studies [18] and clinical-case studies [11,20] were classified as human studies with moderate quality of evidence, while clinical-case studies [19] were classified as studies of low certainty.

The RoB analysis results were identified as a major limitation of the studies [11,18,20], which underestimated the level of certainty. A factor that also affected our assessment was the study group size. A study in which the study group was relatively small was publication [18], in which only 62 patients were included, which affected the overall assessment. A similar problem occurred when assessing the study [19]. The reason was the very small study group (only two patients with asymmetry). In the case of that publication, the overall assessment was also lowered by the RoB analysis, as that study received several Unclear or No responses.

In contrast, the animal study by Liu et al. [21] was classified as a study of low certainty. It was an experimental study that was conducted on zebrafish species. The assessment of certainty was mainly due to both the lack of evidence that specific mutations would cause the same effect in humans and a failure to determine the study group size. A weakness of that study was also the result of the RoB analysis according to the SYRCLE's RoB tool, in which the majority of responses were High Risk or Unclear. However, the thesis of that study was also confirmed in other publications, so it was decided to classify that study as of low certainty. It was the only animal study that was classified in that way.

It may be concluded that the role of the PITX2 gene in the development of mandibular asymmetry was moderately confirmed. The importance of this gene was proved in studies, such as [18,19,21]. In contrast, the study [11] did not detect significant differences in terms of the PITX2 genotype in patients with asymmetry, although a possible interaction between this gene and ENPP1 was identified, whose the impact was proved. In the study [20], the authors attempted to identify the single nucleotide polymorphism (SNPs) of PITX2 gene, especially related to PITX2A and PITX2D isoforms in condylar asymmetry. The authors concluded the exclusion of PITX2 polymorphism in the region of PITX2A and PITX2D.

The findings of the above-mentioned publications suggest the need for further studies, following a properly designed protocol, to definitively confirm the role of this gene in the formation of mandibular asymmetry in humans.

7.2. PITX1

Nicot et al., (2014) and Chung et al., (2017) investigated the impact of PITX1 gene mutations on the formation of mandibular asymmetry during embryogenesis. Both publications are human clinical case studies.

Serious RoB was identified as the main limitation of those studies. The main reason was the failure to identify confounding factors and how to deal with them. The study [11] was classified as a study of moderate certainty. In the case of the article [19], in addition to the results of the RoB analysis, the problem was the very small study group and this led to the classification of that study as of low certainty.

The findings of those studies provided contradictory evidence. Therefore, it can be concluded that the impact of this gene on the formation of mandibular asymmetry has not been confirmed.

7.3. ENPP1

Nicot et al., (2020) [13] and Chung et al., (2017) [11] investigated the impact of ENPP1 on the development of mandibular asymmetry. Both publications are human studies. The article [13] is a cross-sectional study, while the article [11] is a clinical case study. The limitations of the publication [11] were discussed above. On the other hand, the main weakness of the study [13] was the result of the RoB analysis, which was mostly determined by a failure to define confounding factors and how to deal with them. In the study [11], authors were unable to identify significant differences in terms of genotypes or alleles tested for SNPs, although the authors point to a possible link between ENPP1 and ACTN3, the importance of which was proved. It should be concluded that the determination of the significance of ENPP1 in mandibular asymmetry formation requires further research, as the available studies do not unambiguously confirm this thesis.

7.4. ESR1

The impact of ESR1 on asymmetry formation was only investigated in the publication by Chung et al. [11]. Their article is classified as a study of moderate certainty, for the reasons discussed above. However, in the absence of other studies that would support the thesis of that study, its conclusions should be treated with caution. Definitive confirmation of the role of this gene certainly needs further properly designed studies.

7.5. ACTN3

The impact of ACTN3 on the development of mandibular asymmetry was investigated in two publications by Chung et al., (2017) [11] and Nicot et al., (2020) [13]. Both publications were classified as studies of moderate certainty and the reasons for this assessment were outlined above. The findings of those studies were classified as scientific evidence of moderate quality. There were no publications that contradicted the impact of ACTN3 on asymmetry formation. Therefore, it can be assumed that the impact of this gene was confirmed to a moderate degree.

7.6. FINGL1

Shi et al. (2021) [16] investigated the impact of FINGL1 on asymmetry formation. Their publication was a case–control animal study and their study group involved chicks with crossed beaks. Due to the very small study group, the lack of proof that such a mutation would have the same effect in humans and the lack of more studies that would also confirm the thesis, the above mentioned study was classified as a study of very low certainty. For the aforementioned reasons, it should be assumed that the role of FINGL1 in mandibular asymmetry formation was not confirmed and there is a need for more research with a well-designed methodology.

7.7. Sulfotransferase NDST1

In their experimental animal study [23], Yasuda et al., (2010) attempted to find out what role heparin sulfate proteoglycans (HS-PGs) play in the formation of the mandible and temporomandibular joint. For this purpose, they generated mice lacking Golgi-associated N-sulfotransferase 1 (Ndst1) that catalyses sulfation of HS-PG glycosaminoglycan chains. That study was classified as a study of very low certainty, mainly due to the result of the RoB analysis according to the SYRCLE's RoB tool, in which the majority of responses were High Risk or Unclear. That study also lacks both a determination of the study group size and evidence that such a mutation in animals would cause the same effect in humans. Both these factors and the lack of other studies that would support the thesis of that study classified the above-mentioned study in this group. The impact of this gene on the development of mandibular asymmetry has not been confirmed and needs further research.

7.8. YPEL1

Yang Tan et al. [22] investigated the impact of YPEL1 overexpression on the mandible. Their publication is an experimental study that was conducted on chicken embryos. It was classified as a study of very low certainty due to the high RoB found using the SYRCLE's RoB analysis, in which the majority of responses were High risk or Unclear. Despite the relatively large study group (140 chicken embryos), the above mentioned study was classified as a study of very low certainty, also due to the lack of more studies that would support the thesis. The conclusion that YPEL1 affects the development of mandibular asymmetry needs further research.

8. Discussion

In this systematic review, there was a focus on genetic factors that are relevant to the aetiopathogenesis of mandibular asymmetry when the defect arises already in the prenatal period as a result of abnormalities in the morphogenesis of the first and second pharyngeal arches. According to the results of our analysis, most findings were obtained for the impact of PITX2. In addition to the publications presented in this review, a database search also found a publication by Lu et al., (1999) [24]. That study was not included in this review due to the lack of a specific analysis of the impact of PITX2 gene mutation on the development of mandibular asymmetry. However, the authors of the above mentioned study were able to prove that PITX2HD$^{-/-}$ embryos had abnormal development of the maxillary and mandibular facial prominences and cleft palate, which may also indicate an association with mandibular malformation. Due to the paucity of studies concerning the impact of PITX family genes on the development of mandibular asymmetry, it was decided to browse the databases for PITX gene function. While browsing databases, an article by Tran et al., (2021) [25] was found, according to which, based on a review of the available literature, the loss of PITX2 leads to defective morphology of the mandible and maxilla. That article was not included in this review due to the type of publication, a literature review. The authors also emphasised the proven occurrence of PITX2 gene mutations in Axenfeld–Rieger syndrome (ARS). Malformations that occur in the craniofacial area, including underdeveloped jaws, are one of the symptoms of this syndrome. That publication also describes the possible impact of PITX1 gene mutations on mouse development. It was found that mutation of this gene may contribute to the development of abnormal Meckel's cartilage, which may also have an impact on mandibular malformations that occur at a later stage of development. Another publication that includes research on PITX1 is [26]. The above-mentioned article was found while researching databases to find information concerning PITX family genes and their functions. Authors of that study proved that their analysis places the human gene, PTX1, on 5q31, a region associated with Treacher Collins syndrome (TCS). Considering this fact and the craniofacial expression pattern of PITX1 during early development, the association of PITX1 with Treacher Collins syndrome seems highly probable, and consequently its role in the formation of malformations of craniofacial structures.

The study [27] was found while searching databases to find the impact and function of ENPP1. In that article, the authors proved that NPP1 plays important roles in calcium and phosphate regulation, repression of soft tissue mineralisation and in maintaining skeletal structure and function. While looking for more information concerning ENPP1, the article [28] was also found. The authors of that article proved that single-nucleotide polymorphism rs9373000 of ENPP1 presented a statistically significant association with condylar height ratio.

While looking for other studies concerning the impact of ESR1 on the development of mandibular asymmetry, the study [29] was found. According to the authors of that study, the specific genetic variation in ESR1 (rs2234693 and rs9340799) and ESR2 (rs1256049 and rs4986938) represents possible markers for variation in the craniofacial dimensions.

Cunha et al., (2018) investigated the role of ACTN3 in the development of malformations of craniofacial structures. They proved in their study [30] the association of genetic

variants in ACTN3 with a craniofacial skeletal pattern. Arino et al., (2017) [31] also addressed this issue. They proved that condylar growth was altered in KO mice, contributing to significant changes in mandibular morphology.

Although the above mentioned publications were not included in the analysis, they provide information that enables a more detailed understanding of the functions and associations among the different genes analysed. It seems that, apart from the genes mentioned above, other genetic mutations are also worth investigating, which may sometimes affect the growth of the mandibular condyles [32]. Finally, the conducted analysis makes it possible to conclude that the gene whose importance seems to be best confirmed is PITX2.

8.1. Limitations of the Study

Unfortunately, all of the analysed studies revealed lesser or greater limitations that indicate a certain caution regarding the studied associations between the mutation of specific genes and the resulting mandibular asymmetry. The failure to define asymmetry, which was an unambiguous term for all authors, was one of these limitations. The authors of human studies used various methods to classify the defect, using several types of X-rays (radiographs) that have low accuracy. Three-dimensional images, such as CBCT, have a much higher accuracy of measurements and they also enable the quantitative analysis of outcomes obtained. Therefore, the need for research using this way of assessing asymmetry is a logical conclusion.

Another limitation was the use of various testing methods during the gene analysis. For example, Nicot et al. used saliva samples taken from patients in their 2020 study, while in their 2014 study, they studied muscle fibre fragments taken from patients and removed during sagittal osteotomy of the mandible using Epker's method.

Therefore, there is a need for another series of studies in which the method of gene analysis is standardised. According to our analysis, the greatest evidential value lies in the findings of human studies rather than animal studies, which is why we also draw attention to the need for further human studies.

The above mentioned limitations did not allow us to enrich our systematic review with a meta-analysis, which would be the best confirmation of the degree of influence of individual genes on the asymmetry of the mandible.

8.2. Conclusions

- The impact of ACTN3 and PITX2 gene mutations on the mandibular asymmetry formation was confirmed to a moderate degree.
- The determination of the role of Ndst1, YPEL1 and FINGL1 genes in asymmetry formation needs more well-designed studies.
- Most of the available articles that analyse the impact of genes on the development of mandibular asymmetry provide only scientific evidence of moderate-to-low quality.
- The analysis of available articles concerning asymmetrical defects of the mandible revealed a relatively small number of studies that focus on this particular type of defect.
- The studies that demonstrated a higher level of certainty were human cross-sectional studies and human clinical case studies. According to our assessment, the above mentioned studies contributed more to our findings compared to animal studies. Based on the foregoing, it can be concluded that there is a need for further studies of this type concerning the impact of genes in mandibular asymmetry formation.
- In order to be able to finally confirm the degree of influence of individual genes on the formation of mandibular asymmetry, it is necessary to perform a meta-analysis. Currently, this is not possible due to the insufficient number of well-designed original studies with a unified method of assessing asymmetry.

Author Contributions: Conceptualization, B.K. and M.S.; methodology, M.S.; software, A.B.; validation, A.B., B.K. and M.S.; formal analysis, A.B.; investigation, A.B.; resources, A.B.; data curation, A.B. and M.S; writing—original draft preparation, A.B.; writing—review and editing, M.S.; visualiza-

tion, B.K.; supervision, M.S.; project administration, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Evangelista, K.; Teodoro, A.B.; Bianchi, J.; Soares Cevidanes, L.H.; de Oliveira Ruellas, A.C.; Garcia Silva, M.A.; Valladares-Neto, J. Prevalence of mandibular asymmetry in different skeletal sagittal patterns: A systematic review. *Angle Orthod.* **2021**, *92*, 118–126. [CrossRef] [PubMed]
2. Sobieska, E.; Walerzak, M.; Molinska, M. Facial asymmetry—Aetiology, classification and diagnostics based on the literature, Zakład Ortodoncji, Warszawski Uniwersytet Medyczny. *Forum. Ortod.* **2020**, *16*, 138–158. [CrossRef]
3. Vieira, A.R. Orthodontics and Genetics. *Dent. Press J. Orthod.* **2019**, *24*, 92–97. [CrossRef] [PubMed]
4. Obwegeser, H.L. *Mandibular Growth Anomalies*; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 2001.
5. Lee, S.K.; Kim, Y.S.; Oh, H.S.; Yang, K.H.; Kim, E.C.; Chi, J.G. Prenatal development of the human mandible. *Anat. Rec.* **2001**, *263*, 314–325. [CrossRef]
6. Cordero, D.R.; Brugmann, S.; Chu, Y.; Bajpai, R.; Jame, M.; Helms, J.A. Cranial Neural Crest Cells on the Move: Their Roles in Craniofacial Development. *Am. J. Med. Genet. A* **2011**, *155*, 270–279. [CrossRef]
7. Anderson, R.M.; Stottmann, R.W.; Choi, M.; Klingensmith, J. Endogenous bone morphogenetic protein antagonists regulate mammalian neural crest generation and survival. *Dev. Dyn.* **2006**, *235*, 2507–2520. [CrossRef]
8. Mayor, R.; Guerrero, N.; Martinez, C. Role of FGF and noggin in neural crest induction. *Dev. Biol.* **1997**, *189*, 1–12. [CrossRef]
9. Garcia-Castro, M.I.; Marcelle, C.; Bronner-Fraser, M. Ectodermal Wnt function as a neural crest inducer. *Science* **2002**, *297*, 848–851. [CrossRef]
10. Mitsiadis, T.A.; Drouin, J. Deletion of the Pitx1 genomic locus affects mandibular tooth mor-phogenesis and expression of the *Barx1* and *Tbx1* genes. *Dev. Biol.* **2008**, *313*, 887–896. [CrossRef]
11. Chung, K.; Richards, T.; Nicot, R.; Vieira, A.R.; Cruz, C.V.; Raoul, G.; Ferri, J.; Sciote, J.J. ENPP1 and ESR1 genotypes associated with subclassifications of craniofacial asymmetry and severity of tempomandibular disorders. *Am. J. Orthod. Dentofac. Orthop.* **2017**, *152*, 631–645.
12. Nicot, R.; Vieira, A.R.; Raoul, G.; Delmotte, C.; Duhamel, A.; Ferri, J.; Sciote, J.J. ENPP1 and ESR1 genotypes influence temporomandibular disorders development and surgical treatment response in dentofacial deformities. *J. Cranio-Maxillofac. Surg.* **2016**, *44*, 1226–1237. [CrossRef]
13. Nicot, R.; Chung, K.; Vieira, A.R.; Raoul, G.; Ferri, J.; Sciote, J.J. Condyle modeling stability, craniofacial asymmetry and ACTN3 genotypes: Contribution to TMD prevalence in a cohort of dentofacial deformities. *PLoS ONE* **2020**, *15*, e0236425. [CrossRef]
14. Available online: https://jbi.global/critical-appraisal-tools (accessed on 25 January 2022).
15. Ma, L.L.; Wang, Y.Y.; Yang, Z.H.; Huang, D.; Weng, H.; Zeng, X.T. Methodological quality (risk of bias) assessment tools for primary and secondary medical studies: What are they and which is better? *Mil. Med. Res.* **2020**, *7*, 1–11. [CrossRef]
16. Shi, L.; Bai, H.; Li, Y.; Yuan, J.; Wang, P.; Wang, Y.; Ni, A.; Jiang, L.; Ge, P.; Bian, S.; et al. Analysis of DNA Methylation Profiles in Mandibular Condyle of Chicks with Crossed Beaks Using Whole-Genome Bisulfite Sequencing. *Front. Genet.* **2021**, *12*, 680115. [CrossRef]
17. Hooijmans, C.R.; Rovers, M.M.; de Vries, R.B.M.; Leenaars, M.; Ritskes-Hoitinga, M.; Langendam, M.W. SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* **2014**, *14*, 1–9. [CrossRef]
18. Sofyanti, E.; Soegiharto, B.; Trelia, B.; Ilyas, S. Polymorphism in 4'-UTR Region of PITX2 in Vertebral Mandibular Symmetry. *Hiroshima J. Med. Sci.* **2018**, *67*, 120–126.
19. Nicot, R.; Raoul, G.; Horton, M.; Barton, E.; Sciote, J.J. Nodal Pathway Genes are Down-Regulated in Facial Asymmetry. *J. Craniofacial Surg.* **2014**, *25*, e548–e555. [CrossRef]
20. Sofyanti, E.; Boel, T.; Soegiharto, B.; Ilyas, S.; Nainggolan, L.I.; Auerkari, E.I. Exclusion of pituitary homeobox 2 gene polymorphism in vertical mandibular asymmetry patients: A preliminary study. In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bristol, UK, 2018; Volume 130, p. 012016.
21. Liu, Y.; Semina, E.V. *pitx2* Deficiency Results in Abnormal Ocular and Craniofacial Development in Zebrafish. *PLoS ONE* **2012**, *7*, e30896. [CrossRef]
22. Tan, T.Y.; Gordon, C.T.; Miller, K.A.; Amor, D.J.; Farlie, P.G. YPEL1 Overexpression in Early Avian Craniofacial Mesenchyme Causes Mandibular Dysmorphogenesis by Up-Regulating Apoptosis. *Dev. Dyn.* **2015**, *244*, 1022–1030. [CrossRef]
23. Yasuda, T.; Mundy, C.; Kinumatsu, T.; Shibukawa, Y.; Shibutani, T.; Grobe, K.; Minugh-Purvis, N.; Pacifici, M.; Koyama, E. Sulfotransferase Ndst1 is Needed for Mandibular and TMJ. Development. *J. Dent. Res.* **2010**, *89*, 1111. [CrossRef]
24. Lu, M.F.; Pressman, C.; Dyer, R.; Johnson, R.L.; Martin, J.F. Function of Rieger syndrome gene in left±right asymmetry and craniofacial development. *Nature* **1999**, *401*, 276–278. [CrossRef] [PubMed]

25. Tran, T.Q.; Kioussi, C. Pitx genes in development and disease. *Cell. Mol. Life Sci.* **2021**, *78*, 4921–4938. [CrossRef] [PubMed]
26. Crawford, M.J.; Lanctôt, C.; Tremblay, J.J.; Jenkins, N.; Gilbert, D.; Copeland, N.; Beatty, B.; Drouin, J. Human and murine PTX1/Ptxl gene maps to the region for Treacher Collins Syndrome. *Mamm. Genome* **1997**, *8*, 841–845. [CrossRef] [PubMed]
27. Mackenzie, N.C.W.; Zhu, D.; Milne, E.M.; van't Hof, R.; Martin, A.; Quarles, D.L.; Milla, J.L.; Farquharson, C.; MacRae, V.E. Altered Bone Development and an Increase in FGF-23 Expression in Enpp1$^{(-/-)}$ Mice. *PLoS ONE* **2012**, *7*, e32177. [CrossRef]
28. Constant, M.; Nicot, R.; Vieira, A.R.; Raoul, G.; Sciote, J.J.; Ferri, J. Condylar geometry variation is associated with ENPP1 variant in a population of patients with dento-facial deformities. *J. Cranio-Maxillofac. Surg.* **2017**, *45*, 826–830. [CrossRef]
29. Omori, M.A.; Gerber, J.T.; Marañón-Vásquez, G.A.; Matsumoto, M.A.N.; Weiss, S.G.; Nascimento, M.A.; de Souza Araújo, M.T.; Sasso Stuani, M.B.; Nelson-Filho, P.; Scariot, R.; et al. Possible as-sociation between craniofacial dimensions and genetic markers in ESR1 and ESR2. *J. Orthod.* **2020**, *47*, 65–71. [CrossRef]
30. Cunha, A.; Nelson-Filho, P.; Artemio, G.; Vasquez, M.; de Carvalho Ramos, A.G.; Dantas, B.; Sebastiani, A.M.; Silverio, F.; Omori, M.A.; Rodrigues, A.S.; et al. Genetic variants in ACTN3 and MYO1H are associated with sagittal and vertical craniofacial skeletal patterns. *Arch. Oral. Biol.* **2018**, *97*, 85–90. [CrossRef]
31. Arino, F.; Sciote, J.; Houweling, P.; North, K. Effects of Actn3 KO on mandibular condyle microarchitecture and bone morphology. *J. Dent. Res. A* **2017**, *96*, 1769.
32. Paradowska-Stolarz, A. MSX1 gene in the etiology orofacial deformities. *Postepy Hig. Med. Dosw.* **2015**, *69*, 1499–1504.