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P450 oxidoreductase deficiency: A systematic review and meta-analysis of genotypes, phenotypes and their relationships

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Abstract

Context

P450 oxidoreductase deficiency (PORD) is a rare genetic disorder that is associated with significant morbidity. However there has been limited analysis of reported PORD cases.

Objective

To determine, based on the cohort of reported PORD cases, genotype-phenotype relationships for skeletal malformations, maternal virilisation in pregnancy, adrenal insufficiency and disorders of sexual development (DSD).

Data Sources

PubMed and Web of Science from January 2004 to February 2018.

Study Selection

Published case reports/series of patients with PORD. Eligible patients were unique, had biallelic mutations and their clinical features reported.

Data Extraction

Patient data were manually extracted from the text of case reports/series. A malformation score, representing the severity of skeletal malformations, was calculated for each patient.

Data Synthesis

Of the 211 patients published in the literature, 90 patients were eligible for inclusion. Over 60 unique mutations were identified in this cohort. Four groups of mutations were identified, through regression modelling, as having significantly different skeletal malformation scores. Maternal virilisation in pregnancy, reported for 21% of patients, was most common for R457H mutations. Adrenal insufficiency occurred for the majority of patients (78%) and was typically mild, with homozygous

R457H mutations being the least deficient. DSD affected most patients (72%) but were less common for males (46XY) with homozygous R457H mutations.

Conclusions

PORD is a complex disorder with many possible mutations affecting a large number of enzymes. By analysing the cohort of reported PORD cases, this study identified clear relationships between genotype and several important phenotypic features.

Introduction

The enzyme P450 oxidoreductase (POR) contains 680 amino acids and is encoded by the POR gene on chromosome 7 (1). POR transfers electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to 50 microsomal P450 enzymes, which are important in steroidogenesis (e.g. CYP17A1, CYP19A1, CYP21A2), cholesterologenesis (e.g. CYP51A1) and drug metabolism (e.g. CYP3A4), and to several non-P450 enzymes (2).

P450 oxidoreductase deficiency (PORD) is a rare autosomal recessive variant of congenital adrenal hyperplasia (CAH) arising from homozygous or compound heterozygous POR mutations. While the steroid fingerprint was first described in 1985 (3), the genetic defect was not defined for 20 years (4,5). Over 100 cases have now been reported, with most occurring in neonates and children. Patients with PORD have a range of skeletal malformations, glucocorticoid deficiency and disorders of sexual development (DSD). More than 50 different POR mutations have been identified, including missense, nonsense, insertion, deletion, duplication, splice site and frameshift mutations. Homozygous null mutations appear to be lethal (6,7).

A single pair of POR mutations can impair a large number of enzymes that rely on POR for electron transfer. Impairments in enzymes involved in cholesterol synthesis (CYP51A1 and squalene epoxidase) and retinoic acid metabolism (CYP26 isozymes) are believed to cause skeletal malformations (7). Loss of function of CYP17A1 is associated with DSD, specifically male undervirilisation (due to decreased androgen production) and female virilisation (due to utilisation of a backdoor pathway for androgen synthesis in fetal life) (2,7,8). Impairments to placental CYP19A1 may result in virilisation of the pregnant mother (8). Although one pair of POR mutations can impair all of these enzymes, each enzyme is typically affected to a different extent (depending on the locations of the POR mutations), resulting in high variability of the clinical presentation of PORD.

Skeletal malformations in PORD affect the face (midface hypoplasia), cranium (craniosynostosis), hands and feet (arachnodactyly, talipes), large joints (radiohumeral synostosis), femurs (bowing, fractures), and other areas (e.g. scoliosis, pectus excavatum). The severity of these features can be assessed using a scoring system developed by Krone et al. (7), which used data on 30 patients. However, there has been limited analysis of the relationship between different pairs of POR

mutations and skeletal malformation severity. Two previous studies mainly focused on patients with certain racial backgrounds (Japanese and Caucasian) only (6,7), limiting the range of mutations for analysis. The aims of this study were to analyse skeletal malformations in the entire published PORD cohort and to analyse maternal virilisation in pregnancy, adrenal insufficiency, hormone concentrations, blood pressure and DSD, with particular reference to genotype-phenotype relationships.

Methods

Case identification

PORD case reports and series were identified by systematic PubMed and Web of Science searches conducted on 9 March 2018. A broad search term was used for both databases: "P450 oxidoreductase" AND ("deficiency" OR "deficient" OR "mutation"). As shown by Figure 1, 382 articles were identified, with a further 3 articles identified from scanning reference and citation lists. After duplicate articles were removed, 259 articles were screened for eligibility. Articles were excluded if they were published before 2004 (i.e. before PORD was formally identified) or if they did not contain any clinical data on patients. Forty-seven articles remained after exclusions, containing a total of 236 patients, with 211 of these having biallelic POR mutations (confirmed PORD cases). Patients were excluded if: i) insufficient clinical information was provided, ii) mutations were identified in other genes associated with dysmorphic features, or iii) the patient had already been described in another article (i.e. the patient was a duplicate). At the end of this process, 90 unique patients with PORD were identified from published articles.

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed (9). Quality assessment tools such as the Newcastle-Ottawa Scale (10) were not considered appropriate to be used since they were not developed to evaluate case reports or series. Table 1 summarises the number of patients that were included in and/or excluded from each article.

Data Collection

For each of the 90 uniquely identified PORD patients, all supporting text was reviewed in the corresponding published article and relevant clinical data were collected and organised into a dataset. Specifically, data were collected on nationality, karyotype, mutation type and clinical features relating to blood pressure, hormone concentrations, skeletal malformations, maternal virilisation in pregnancy, adrenal insufficiency and DSD. All collected data were reviewed to ensure consistent terminology (e.g. club foot and talipes were recorded as the same feature). As patients with PORD may present with DSD, sex was assessed based on karyotype, which was reported for 81 patients.

Hormone concentrations

Serum hormone concentrations and normal reference ranges were collected from reported data for each patient, where available, for the following hormones: progesterone, pregnenolone, 17-hydroxyprogesterone (17OHP), corticosterone, deoxycorticosterone (DOC), dehydroepiandrosterone (DHEA), cortisol, aldosterone and androstenedione. Each serum concentration was classified as low, normal or high based on the reported normal reference range.

Malformation score calculation

Each individual skeletal malformation was categorised into one of six "domains" of skeletal malformations in PORD (7): 1) midface hypoplasia, 2) craniosynostosis, 3) hand and feet malformations, 4) large joint synostosis, 5) femoral bowing or 6) additional malformations. For each patient, a score was calculated for each domain using the criteria developed by Krone et al. (7). Finally, for each patient, the domain scores were summed to give the total "malformation score" (maximum score of 16). A higher malformation score indicated greater skeletal malformation severity.

A validation study was performed to assess the appropriateness of calculating the malformation score based only on data from published reports. This consisted of a sub-analysis of data on 16 patients. For these patients, the published article provided details of the skeletal

abnormalities as well as the "true" malformation score (as calculated by the articles' authors using Krone et al.'s (7) criteria) (7,36,38).

One article in the data collection (Homma et al. (18), which contained 7 unique PORD patients), did not provide data on skeletal malformations at a domain level, but instead reported whether the patient's overall skeletal malformations were absent, mild, or severe. In order to utilise these data, the categories were mapped to malformation scores of 0, 2 and 12, respectively. This mapping was based on Krone et al.'s (7) system, where absent, mild and severe malformations were defined by score ranges of 0, 1-4 and 9-16, respectively.

Demographic characteristics of patients were evaluated using Chi squared tests for categorical variables and t-tests for continuous variables. When the median was used, range was displayed. Where variances were unequal, the Welch statistic was used to determine significance. A p-value < 0.05 was considered significant.

Genotype-phenotype analyses

Genotype-phenotype analyses were undertaken for different pairs of POR mutations and i) skeletal malformation severity, ii) maternal virilisation in pregnancy, iii) adrenal insufficiency and iv) DSD.

Skeletal malformations

For the skeletal anomalies, an examination of the relationship between each pair of POR mutations and the calculated malformation score for each patient was undertaken. Pairs of POR mutations with similar malformation scores were grouped together by: i) categorising each individual mutation as an R457H, A287P, apparent null (frameshift, nonsense, deletion, insertion, duplication and splice site mutations – that is, major loss of function mutations) or "other" mutation (missense mutations other than R457H and A287P), ii) forming groups based on each possible pairing of these categories, and iii) comparing the sample means of the malformation scores of each group. Malformation scores were also reviewed manually, and it was noted that four missense mutations were associated with low malformation scores (C569Y, G539R, L577R, Y326D).

Four distinct groups (groups A-D) were identified (Figure 2), which contained 84% of patients in the cohort (76/90 patients). The remaining 16% were excluded from further analysis of skeletal anomalies. Confidence intervals were calculated for the mean malformation scores of groups A-D by fitting the data with a zero-inflated negative binomial (ZINB) model and performing a parametric bootstrap. The ZINB model accommodated for groups having several malformation scores of zero, which occurred when patients had no skeletal malformations. Groups with non-overlapping 95% confidence intervals for the mean malformation score were regarded as significantly different (53). The modelling was conducted in R using the pscl package (54-56).

Maternal virilisation

For the evaluation of maternal virilisation in pregnancy, data on POR mutation pairs and the presence of maternal virilisation were collected. Pairs of POR mutations were divided into eight groups (based on different combinations of R457H, A287P and null mutations): 1) R457H/R457H, 2) R457H/other (where "other" denotes missense mutations other than R457H and A287P), 3) R457H/null, 4) A287P/A287P, 5) A287P/other, 6) A287P/null, 7) other/null, 8) remainder. Chi-squared tests were used to compare the proportion of cases of maternal virilisation in pregnancy in each group.

Adrenal insufficiency

For the evaluation of adrenal insufficiency, data on POR mutation pairs and peak cortisol after ACTH stimulation were collected. Cortisol concentrations were converted to SI units (nmol/L). Adrenal insufficiency was defined by a peak cortisol after ACTH stimulation of less than 500 nmol/L (57). Pairs of POR mutations were divided into two groups: i) both missense mutations or ii) one or more null mutations present. The mean peak cortisol concentration was compared between the two groups using a t-test. Within the missense mutation group, the mean peak cortisol concentration of R457H/R457H mutations was compared to other missense combinations using a t-test.

Disorders of sexual development

For the evaluation of DSD, data on POR mutation pairs and the presence of DSD were collected. Male (46XY) and female (46XX) datasets were constructed (based on karyotype) and the POR mutation pairs within each dataset were divided into eight groups: 1) R457H/R457H, 2) R457H/other, 3) R457H/null, 4) A287P/A287P, 5) A287P/other, 6) A287P/null, 7) other/null, 8) remainder. Chi-squared/Fisher tests were used to compare the proportion of DSD cases between males (46XY) and females (46XX) and between each mutation group. The incidence of ovarian cysts was also calculated for female (46XX) patients.

Results

Patient demographics

Of the 90 unique patients in the cohort, 42 were collected from four larger studies (5,7,16,18), while the remaining 48 patients were collected from 30 smaller studies. Twenty-four nationalities were represented in the cohort, with Japan (29%), United States (7%), Britain (6%) and Germany (6%) being the most common. Eighteen patients had no nationality reported; however, of these, 8 were Caucasian and 4 were Bedouin. The majority (86%) of patient reports were published before 2014. Blood pressure was reported for 15 patients, with 12 patients (80%) being normotensive and 3 patients (20%) being hypertensive. Karyotype evidence, available in 81 patients, showed that the number of affected females (46XX, n=46) and males (46XY, n=35) were not significantly different ($\chi^2_1 = 1.49$, $p > 0.05$). There was also no significant difference between the malformation scores of males (46XY) and females (46XX).

Incidence of different skeletal malformations

Data on individual skeletal malformations was available for 83 patients. A skeletal malformation was reported in 84% (n=70) of patients: 71% (n=59) had midface hypoplasia, 65% (n=54) had craniosynostosis, 61% (n=51) had hand and feet malformations, and 69% (n=57) had large joint synostosis. These proportions were not significantly different from one another. However, they were

significantly different to the proportion of patients with femoral bowing (25%, n=21, $p < 0.001$). Most patients (81%, n=67) had skeletal malformations across multiple domains (median 4, range 0-6). All 6 domains were affected in only 10% (n=8) of patients. Figure 3 shows the incidences of these skeletal malformations stratified by severity (using Krone et al.'s (7) classification). The most frequently reported feature having the greatest severity was bilateral fixed radiohumeral synostosis (n=40, 48%).

Incidence of different POR mutations

Data on the pair of POR mutations were available for all 90 patients. Of these, 57 pairs were uniquely different. Corresponding to the 90 pairs of POR mutations, there were 180 individual POR mutations in total (one for each allele), of which 63 were uniquely different. Seventy-two percent (n=129) were missense mutations and 28% (n=51) were apparent null mutations. The most common individual mutations were R457H (n=45, 25%) and A287P (n=43, 24%) (Figure 4). The proportions of missense (72%) and null mutations (28%) were not significantly different to theoretical proportions of 67% and 33%, respectively, given that PORD is an autosomal recessive disorder with homozygous null mutations assumed to be lethal.

Hormone concentrations

Relative to normal reference ranges, patients in the cohort had high serum concentrations of progesterone (100%, 18/18 patients), pregnenolone (100%, 3/3 patients), 17OHP (96%, 47/49 patients), corticosterone (83%, 5/6 patients) and DOC (70%, 7/10 patients). Serum concentrations were variable for DHEA (10 patients: 5 low, 5 normal, 0 high), baseline cortisol (34 patients: 3 low, 30 normal, 1 high), aldosterone (13 patients: 1 low, 10 normal, 2 high) and androstenedione (24 patients: 8 low, 12 normal, 4 high).

Validation study

The validation study, focusing on skeletal malformations, demonstrated that 11 out of 16 patients had a calculated malformation score (based on data presented in published articles) that was equal to the true score (as stated by the articles' authors). The scores deviated by ± 2 for two patients (Figure 5).

In these cases, it appears the researchers used more information in their calculation than was explicitly stated in the articles. However, as these differences were relatively small (malformation score ranges from 0-16), and they were approximately symmetric with no obvious bias, the calculated malformation score appeared to be a good estimator of the true score.

Genotype-phenotype analyses

Skeletal malformations

Skeletal malformation analysis was done in 10 patients in group A, 12 in B, 16 in C and 38 in D. Eight of the 10 patients (80%) in group A, and 3 of the 12 patients (25%) in group B, had malformation scores of zero (no skeletal abnormality). In contrast all patients (100%) in group D had skeletal malformations in multiple domains (median number of affected domains = 5, range = 2-6). The mean malformation score of groups A-D increased from 0.8, 3.5, 7.5 to 9.2, respectively.

The fit of the ZINB model was deemed to be appropriate based on Pearson's goodness-of-fit test. The coefficients in the zero-inflation component of the model were statistically significant (p-value < 0.001). The 95% confidence intervals for groups A, B and D demonstrated that there was a significant difference between the group means (Figure 6).

Maternal virilisation

Maternal virilisation was reported for 21% (n=19) of mothers during their pregnancy. This occurred with the highest incidence when one or more of the mutations was R457H (Figure 7). The incidence was higher for R457H/R457H mutations (67%) than R457H/null mutations (27%), but this difference was not significant. Maternal virilisation also occurred in 22% and 27% of cases with A287P/A287P and other/null mutations, respectively. Outside of these groups, maternal virilisation in pregnancy was rare or unobserved.

Adrenal insufficiency

Peak cortisol after ACTH stimulation was reported for 54% (n=49) of patients. Adrenal insufficiency, based on peak cortisol less than 500 nmol/L, was present for 78% (n=38) of patients. The majority (76%, n=29) of these patients had peak cortisol concentrations between 250 and 500 nmol/L. The mean peak cortisol for patients with a pair of missense mutations (415 nmol/L, n=21) was not significantly different to patients with one or more null mutations (435 nmol/L, n=28). However, the mean peak cortisol for patients with R457H/R457H mutations (520 nmol/L, n=8) was significantly different to patients with other missense mutations (351 nmol/L, n=13), as suggested by Figure 8.

Disorders of sexual development

DSD were present for 72% (n=65) of patients. Of the 81 patients where a karyotype was reported (46 females (46XX) and 35 males (46XY)), DSD were present for 78% (n=36) of females (46XX) and 60% (n=21) of males (46XY). These proportions were not significantly different. For both sexes, there was no significant difference in the proportion of patients with DSD across the following groups: 1) R457H/R457H, 2) R457H/other, 3) R457H/null, 4) A287P/A287P, 5) A287P/other, 6) A287P/null, 7) other/null, 8) remainder. However, for patients with R457H/R457H mutations, the proportion of females (46XX) (7/7, 100%) and males (46XY) (2/5, 40%) with DSD were significantly different (Fisher test, p-value < 0.05). Ovarian cysts were reported for 39% (n=18) of females (46XX) and occurred across a range of mutations.

Discussion

By using data from more than 30 articles on 90 individual patients from 24 nationalities, this study reports the distribution of skeletal abnormalities, maternal virilisation, adrenal insufficiency and DSD in the largest number of unique PORD patients to date. The results demonstrate that there is no specific skeletal anomaly, or group of anomalies, that are characteristic of PORD and that malformations can be either widespread or localised to particular parts of the skeleton. Malformations affect the face, cranium, large joints, and hands and feet in equal proportions. The severity of skeletal

malformations varies according to the distribution of POR mutation pairs. Maternal virilisation in pregnancy is most common among patients with R457H mutations. Adrenal insufficiency appears to be similar for null and missense mutations, although R457H/R457H mutations are associated with milder adrenal insufficiency. DSD were common in patients with PORD, across all types of mutations, but were less common in males (46XY) with R457H/R457H mutations. Statistical modelling demonstrated a number of new genotype-phenotype relationships, recognition of which may assist in inferring the underlying genotype in affected children, especially in situations where genotype analysis may not be available.

PORD patients were found to have a characteristic hormonal profile. Serum concentrations were typically elevated for progesterone, pregnenolone, 17OHP, corticosterone and DOC, but were variable for DHEA, baseline cortisol, aldosterone and androstenedione. These results are consistent with previous reports (58). Patients were typically normotensive at the time of investigation, but 20% of patients were mildly hypertensive, most likely secondary to elevated DOC (7). Recognition of these characteristics may be helpful to clinicians when considering a diagnosis of PORD.

Skeletal malformations were identified in 84% of patients with PORD, which is consistent with previous reports (59). This study showed that the severity of these anomalies was different for different pairs of POR mutations. The least severe skeletal malformations were associated with a select group of missense mutations (C569Y, G539R, L577R, Y326D). This was followed by homozygous R457H mutations, which were, in turn, less severe than other combinations of R457H and A287P mutations. This provides evidence refuting the earlier assumption that R457H mutations (including homozygotes) tended to produce more severe skeletal malformations than A287P mutations (25). In PORD, the skeletal malformations are thought to arise from impairments to CYP51A1, squalene epoxidase and CYP26 isozymes, although the pathophysiology of skeletal phenotypes is not yet fully understood. The skeletal malformations of PORD are indistinguishable from those of Antley-Bixler syndrome (ABS) (5) but, unlike PORD, ABS does not present with disordered steroidogenesis or DSD.

Maternal virilisation in pregnancy was reported for 21% of mothers, with the highest incidence found when the infant had an R457H mutation. This is consistent with studies showing that

the R457H mutation abolishes the activity of aromatase (CYP19A1) (60), which interferes with the conversion of fetal adrenal derived DHEA and DHEAS to oestrogen. Three cases of maternal virilisation in pregnancy also occurred for I444fsX449 mutations, suggesting that this frameshift mutation also diminishes aromatase activity. The A287P mutation has little effect on aromatase activity (supporting activity remains about 100%) (6), and maternal virilisation in pregnancy was less commonly observed for this mutation.

Adrenal insufficiency was present in the majority of patients with PORD (78%). Most cases were mild, with severe cases being infrequent. As such, many patients would be asymptomatic of adrenal insufficiency under normal conditions, but may not produce sufficient cortisol when exposed to physiological stress such as systemic infection. This study, which is the largest analysis of adrenal insufficiency in PORD, found no significant difference between most genotypes, except for R457H/R457H mutations which were associated with milder adrenal insufficiency. The assertion that adrenal insufficiency cannot be predicted by genotype (7) appears to be true in most cases, but not all.

DSD were present for 72% of patients, which is consistent with previous reports (59). For R457H/R457H mutations, DSD were less common in males (46XY) than females (46XX), suggesting that androgen synthesis is mildly decreased in males (46XY) but markedly increased in females (46XX) (6). The latter may be due to mutant POR having high residual activity in the backdoor pathway for androgen synthesis in fetal life (7). Ovarian cysts were reported for 39% of females (46XX) and occurred across a range of mutations. Ovarian cysts in PORD are thought to arise from two mechanisms: i) impaired production of oestrogen resulting in upregulation of gonadotropins, and ii) impaired production of meiosis-activating sterol, which is important for meiotic resumption and oocyte maturation (8).

Although the genotype-phenotype relationships in PORD are complex, the results from this study may help to predict a patient's genotype from their physical findings and may be beneficial in certain clinical settings. For example, a Japanese patient with minor skeletal abnormalities suggests homozygous R457H mutations. This is considerably more likely if the mother was virilised during pregnancy. Alternatively, a non-Japanese patient with minor skeletal malformations suggests a pair of missense mutations involving C569Y, G539R, L577R, Y326D.

This study has two main limitations. Firstly, it used data extracted from case reports, rather than clinical review of affected patients. As such, it relies on accurate and complete descriptions being provided in each report. While the results are supported by the findings of the validation study, some inaccuracies may be present. Secondly, the cohort of patients reviewed in this study may not be representative of the entire range of abnormalities in patients with PORD. Severe cases of PORD are likely to be detected and diagnosed, whereas less severe cases may go unnoticed (25). Indeed, Miller et al. (1) suggested that PORD may actually be fairly common, but that most of the affected patients have mutations that have minimal or no impact. In addition, patients from underdeveloped areas may not be fairly represented in the cohort (due to a lack of reporting) and this may be contributing to an apparent overabundance of R457H and A287P mutations (from Japanese and Caucasian populations). Given these issues, the results from this study are more generalisable to patients who have historically been diagnosed with PORD and treated. However, given that the evidence base for this disorder is affected by duplications of cases (59), the identification of only unique cases for analysis strengthens the new information provided by this study.

The results of this study suggest at least two areas of future research. Firstly, there can be considerable variation in the skeletal phenotype for the same pair of POR mutations. For example, Homma et al. (18) describe two R457H homozygotes with no skeletal anomalies (malformation score 0), while But et al. (28) describe one R457H homozygote with severe skeletal malformations (malformation score 9). This difference may be due to the presence of undetected mutations on the POR gene or an unidentified factor that also contributes to the skeletal phenotype. Indeed, Burkhard et al. (59) suggested that other genes may contribute to the physical manifestations of PORD. An analysis of patients with the same POR mutations but different phenotypes may be helpful in further understanding this issue. Secondly, rather than performing a genotype-phenotype analysis using an overall malformation score (as done in this study), further analysis could instead focus on specific skeletal features (e.g. radiohumeral synostosis). This may assist in determining the reason for the presence of certain features in some patients but not others.

In conclusion, this study identified and collected data on 90 unique PORD patients and demonstrated that PORD can cause a number of malformations. The presentation of PORD in patients

appears to be highly variable, possibly leading to missed or delayed diagnoses (48). Genotype-phenotype relationships for skeletal malformations, maternal virilisation in pregnancy, adrenal insufficiency and DSD were identified. Further analysis of this type, using data on the other abnormalities found in PORD patients, may assist in the elucidation of this complex and highly variable disorder.

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Table 1 Number of patients included and/or excluded from each article in the data collection.

Figure 1 Flow-chart demonstrating the PubMed and Web of Science search strategy for identifying patients with PORD.

Figure 2 Grouping of pairs of POR mutations used in the analysis of skeletal malformations.

Figure 3 Frequency of patients with different skeletal malformations (stratified from lowest to highest severity) in a sample of 83 patients.

Figure 4 Frequency of different POR mutations in the cohort of 90 patients (180 alleles).

Figure 5 Differences between the calculated and true malformation scores in a sample of 16 patients.

Figure 6 Mean malformation score and 95% confidence interval for groups A-D. The shaded regions correspond to mild (lightest), moderate and severe (darkest) malformations, respectively. Group A: pairs of missense mutations involving C569Y, G539R, L577R, Y326D, but not R457H or A287P. Group B: R457H/R457H. Group C: (R457H/A287P) / Other. Group D: (R457H/A287P) / Null.

Figure 7 Frequency of maternal virilisation in pregnancy for different pairs of POR mutations.

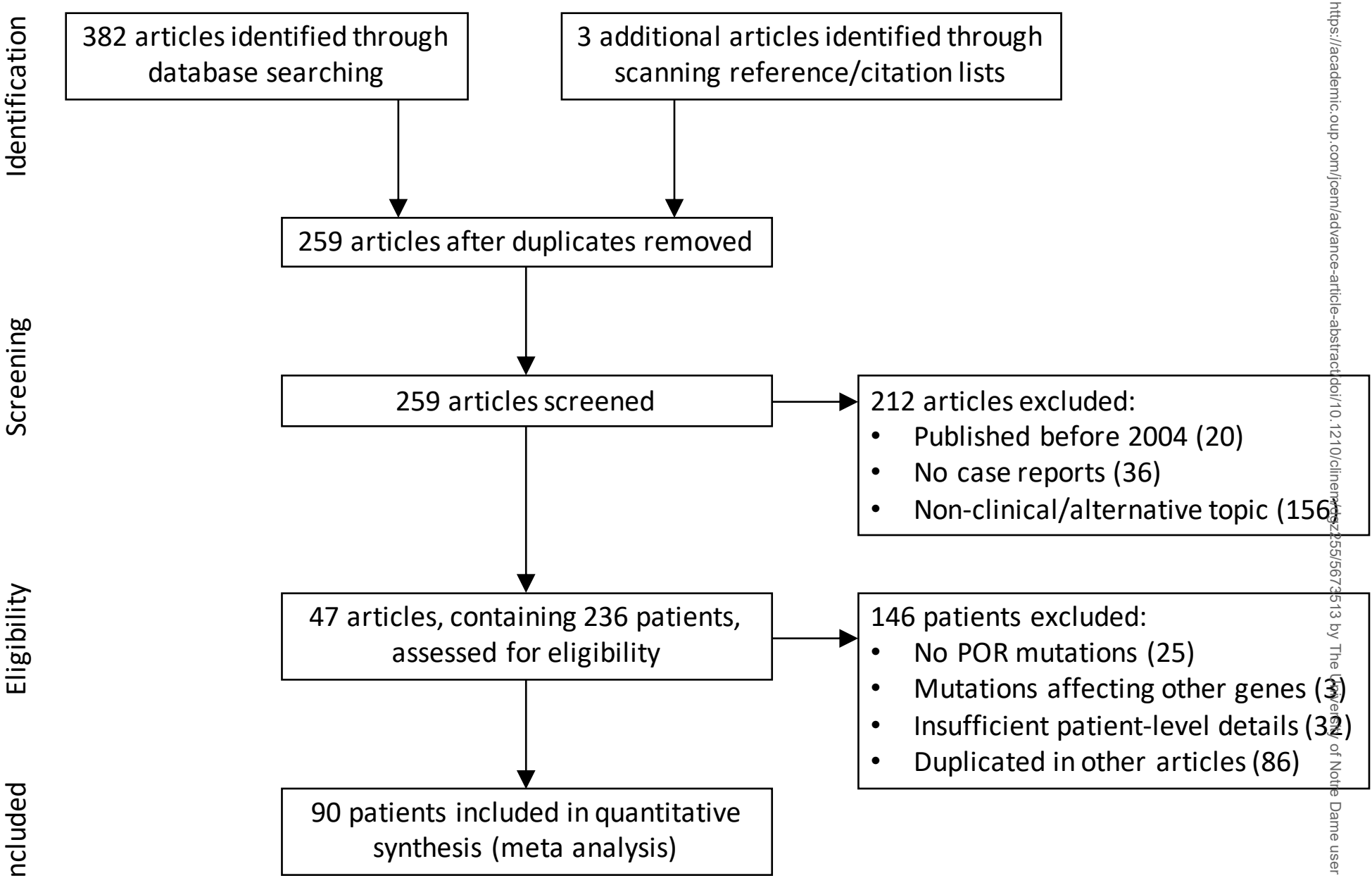
Figure 8 Peak cortisol after ACTH stimulation for R457H/R457H mutations compared to other missense combinations.

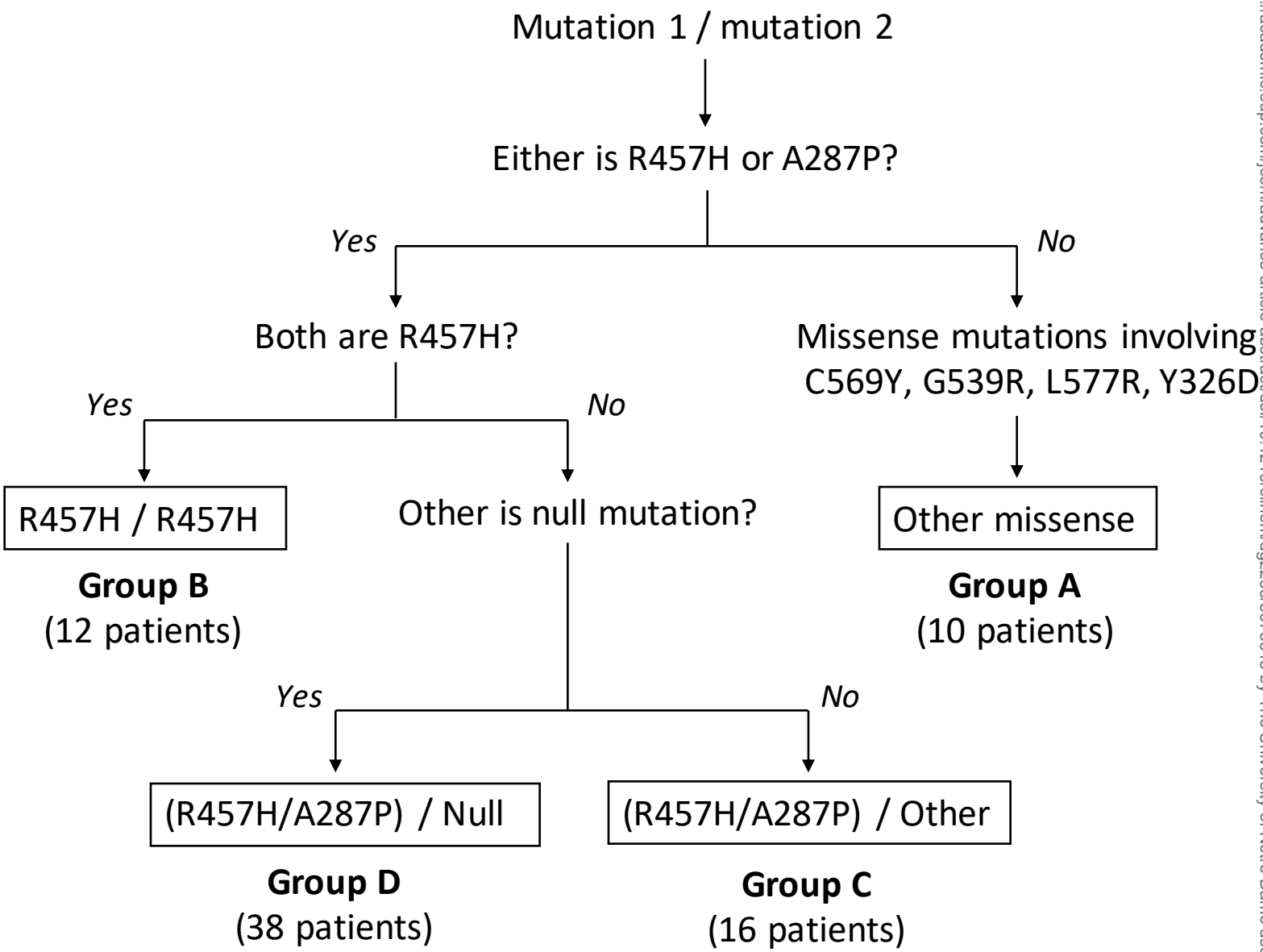
Table 1

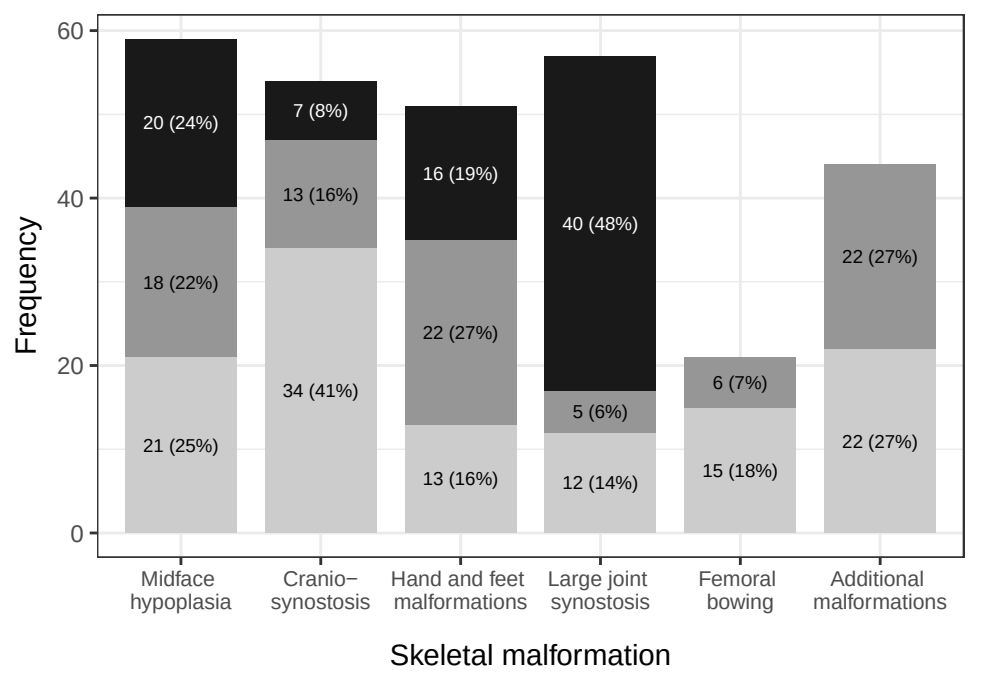
		Exclusions				
Reference	**Number of patients**	**Duplicates**	**Lacking patient data**	**No POR mutations**	**Other mutant genes**	**Included patients**
Fluck, Tajima, Pandey, Arlt, Okuhara, Verge, Jabs, Mendonca, Fujieda, Miller (4)	4	0	0	1	0	3
Arlt, Walker, Draper, Ivison, Ride, Hammer, Chalder, Borucka-Mankiewicz, Hauffa, Malunowicz, Stewart, Shackleton (11)	3	0	0	0	0	3
Adachi, Tachibana, Asakura, Yamamoto, Hanaki, Oka (12)	2	0	0	0	0	2
Wudy, Hartmann, Draper, Stewart, Arlt (13)	1	0	0	0	0	1
Shackleton, Marcos, Arlt, Hauffa (14)	2	2	0	0	0	0
Shackleton, Marcos, Malunowicz, Szarras-Czapnik, Jira, Taylor, Murphy, Crushell, Gottschalk, Hauffa, Cragun, Hopkin, Adachi, Arlt (15)	6	6	0	0	0	0
Huang, Pandey, Agrawal, Reardon, Lapunzina, Mowat, Jabs, Van Vliet, Sack, Fluck, Miller (5)	32	0	0	17	1	14
Fukami, Horikawa, Nagai, Tanaka, Naiki, Sato, Okuyama, Nakai, Soneda, Tachibana, Matsuo, Sato, Homma, Nishimura, Hasegawa, Ogata (16)	10	0	0	2	0	8
Fukami, Hasegawa, Horikawa, Ohashi, Nishimura, Homma, Ogata (17)	3	0	0	0	0	3
Homma, Hasegawa, Nagai, Adachi, Horikawa, Fujiwara, Tajima, Takeda, Fukami, Ogata (18)	22	15	0	0	0	7
Williamson, Arlt, Shackleton, Kelley, Braddock (19)	1	0	0	0	0	1
Scott, Gomes, Huang, Van Vliet, Miller (20)	1	0	0	0	1	0
Dhir, Ivison, Krone, Shackleton, Doherty, Stewart, Arlt (21)	11	0	11	0	0	0
Hershkovitz, Parvari, Wudy, Hartmann, Gomes, Loewental, Miller (22)	4	0	0	0	0	4
Nakamura, Adachi, Machida, Okuzumi (23)	3	3	0	0	0	0
Ko, Cheon, Kim, Yoo (24)	1	0	0	0	0	1
Fukami, Nishimura, Homma, Nagai, Hanaki, Uematsu, Ishii,	35	23	12	0	0	0

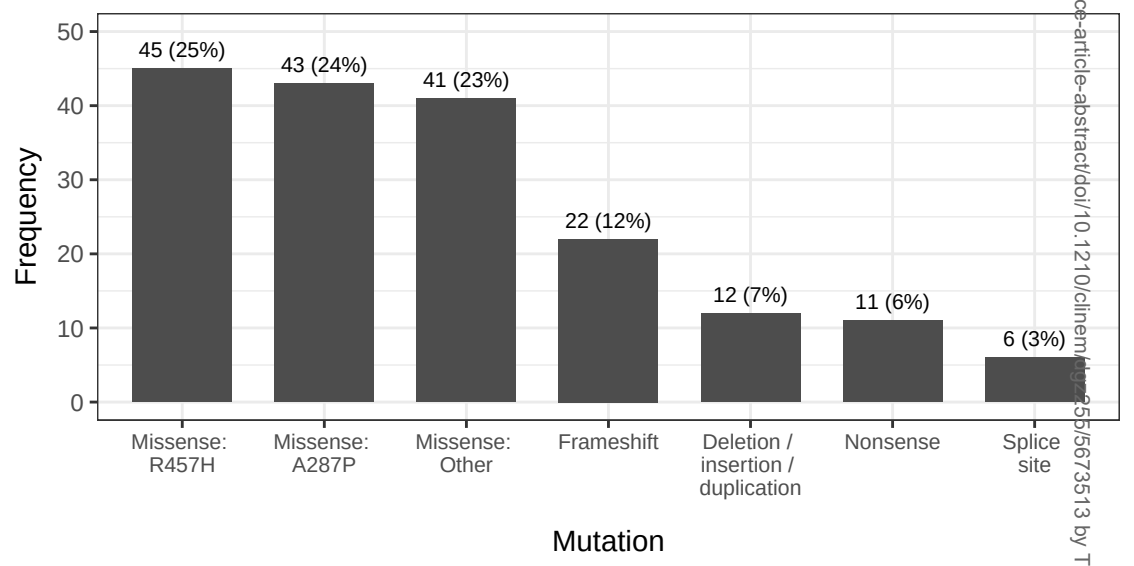
Numakura, Sawada, Nakacho, Kowase, Motomura, Haruna, Nakamura, Ohishi, Adachi, Tajima, Hasegawa, Hasegawa, Horikawa, Fujieda, Ogata (6)						
Sahakitrungruang, Huang, Tee, Agrawal, Russell, Crock, Murphy, Migeon, Miller (25)	4	0	0	0	0	4
Iijima, Ohishi, Ohzeki (26)	1	0	0	0	0	1
Idkowiak, Malunowicz, Dhir, Reisch, Szarras-Czapnik, Holmes, Shackleton, Davies, Hughes, Krone, Arlt (27)	1	0	0	0	1	0
But, Lo, Shek, Tse, Lam (28)	1	0	0	0	0	1
McGlaughlin, Witherow, Dunaway, David, Anderson (29)	2	0	2	0	0	0
Fukami, Nagai, Mochizuki, Muroya, Yamada, Takitani, Ogata (30)	2	0	2	0	0	0
Tomalik-Scharte, Maiter, Kirchheiner, Ivison, Fuhr, Arlt (31)	1	1	0	0	0	0
Herkert, Blaauwwiekel, Hoek, Veenstra-Knol, Kema, Arlt, Kerstens (32)	1	0	0	0	0	1
Idkowiak, O'Riordan, Reisch, Malunowicz, Collins, Kerstens, Kohler, Graul-Neumann, Szarras-Czapnik, Dattani, Silink, Shackleton, Maiter, Krone, Arlt (8)	7	1	0	0	0	6
Fluck, Mallet, Hofer, Samara-Boustani, Leger, Polak, Morel, Pandey (33)	2	0	2	0	0	0
Soneda, Yazawa, Fukami, Adachi, Mizota, Fujieda, Miyamoto, Ogata (34)	3	3	0	0	0	0
Krone, Reisch, Idkowiak, Dhir, Ivison, Hughes, Rose, O'Neil, Vijzelaar, Smith, MacDonald, Cole, Adolphs, Barton, Blair, Braddock, Collins, Cragun, Dattani, Day, Dougan, Feist, Gottschalk, Gregory, Haim, Harrison, Olney, Hauffa, Hindmarsh, Hopkin, Jira, Kempers, Kerstens, Khalifa, Kohler, Maiter, Nielsen, O'Riordan, Roth, Shane, Silink, Stikkelbroeck, Sweeney, Szarras-Czapnik, Waterson, Williamson, Hartmann, Taylor, Wudy, Malunowicz, Shackleton, Arlt (7)	30	12	1	4	0	13
Puiu, Pienar, Chirita, Arghirescu, Popa, Micle (35)	1	0	0	0	0	1

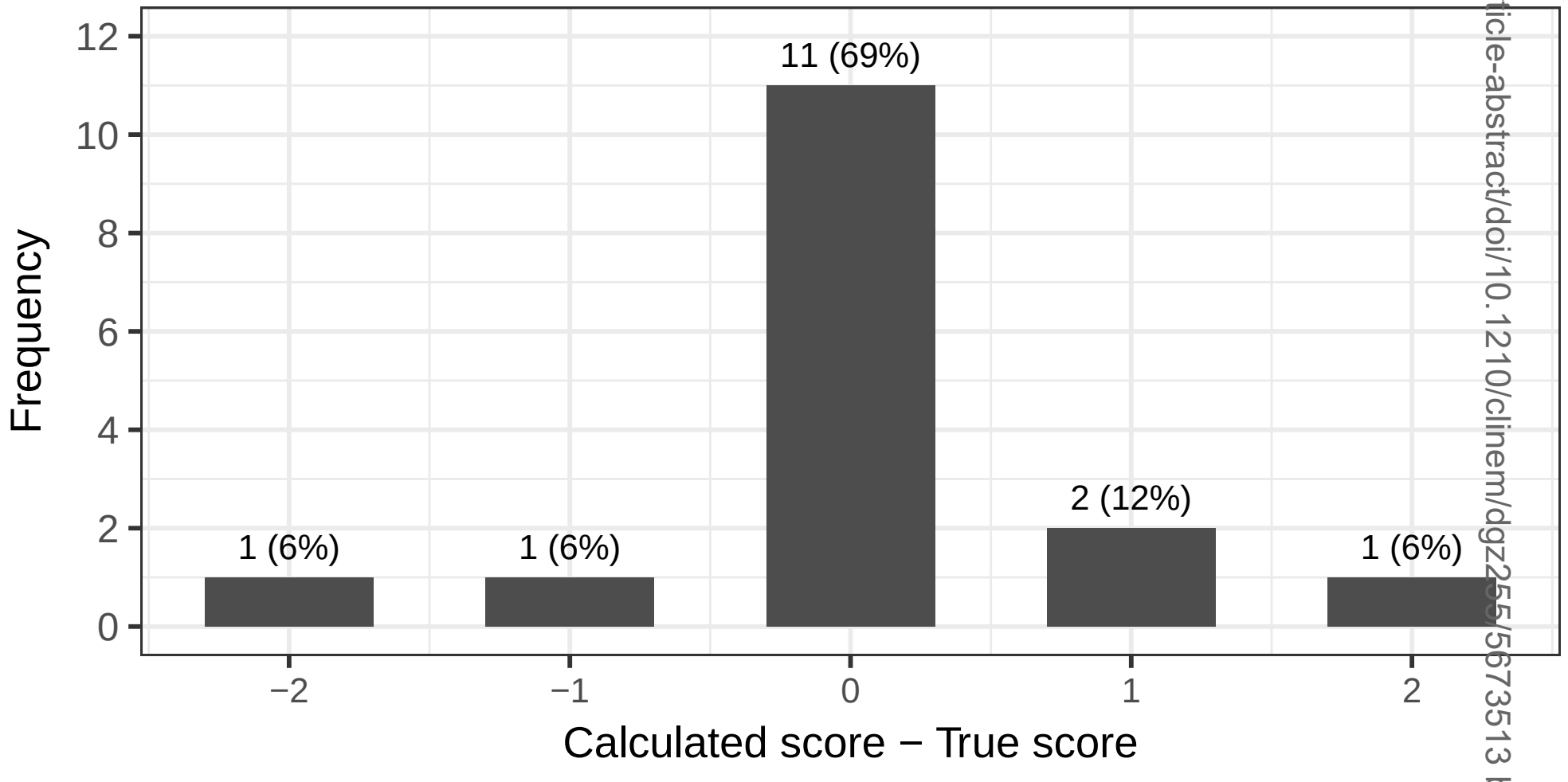
Guaragna-Filho, Castro, Carvalho, Coeli, Ferraz, Petroli, Mello, Sewaybricker, Lemos-Marini, D'Souza-Li, Miranda, Maciel-Guerra, Guerra-Junior (36)	1	0	0	0	0	1
Sanchez-Garvin, Albaladejo, Ezquieta, Corripio (37)	1	0	0	0	0	1
Reisch, Idkowiak, Hughes, Ivison, Abdul-Rahman, Hendon, Olney, Nielsen, Harrison, Blair, Dhir, Krone, Shackleton, Arlt (38)	20	19	0	0	0	1
Boia, Popoiu, Puiu, Stanciulescu, David (39)	1	1	0	0	0	0
Oldani, Garel, Bucourt, Carbillon (40)	1	0	0	0	0	1
Ghazle, Newcomb (41)	1	0	0	0	0	1
Koika, Armeni, Georgopoulos (42)	1	0	0	0	0	1
Parween, Roucher-Boulez, Fluck, Lienhardt-Roussie, Mallet, Morel, Pandey (43)	1	0	0	0	0	1
Bonamichi, Santiago, Bertola, Kim, Alonso, Mendonca, Bachega, Gomes (44)	1	0	0	0	0	1
Tzetis, Konstantinidou, Sofocleous, Kosma, Mitrakos, Tzannatos, Kitsiou-Tzeli (45)	3	0	2	0	0	1
Nakanishi, Yamashita, Miyamoto, Takeguchi, Furuya, Matsuo, Tanahashi, Kawamura, Sengoku (46)	1	0	0	0	0	1
Woo, Ko, Shin, Yang (47)	1	0	0	0	0	1
Bai, Li, Wang (48)	1	0	0	0	0	1
Song, Wang, Chen, Zhu, Sun (49)	2	0	0	1	0	1
Khadilkar, Jagtap, Lila, Bandgar, Shah (50)	1	0	0	0	0	1
Lantigua, Rubio, Rodriguez-Buritica, Khan, Yafi (51)	1	0	0	0	0	1
Oh, Song, Park, Jang, Ki, Kim (52)	1	0	0	0	0	1
					Total	90

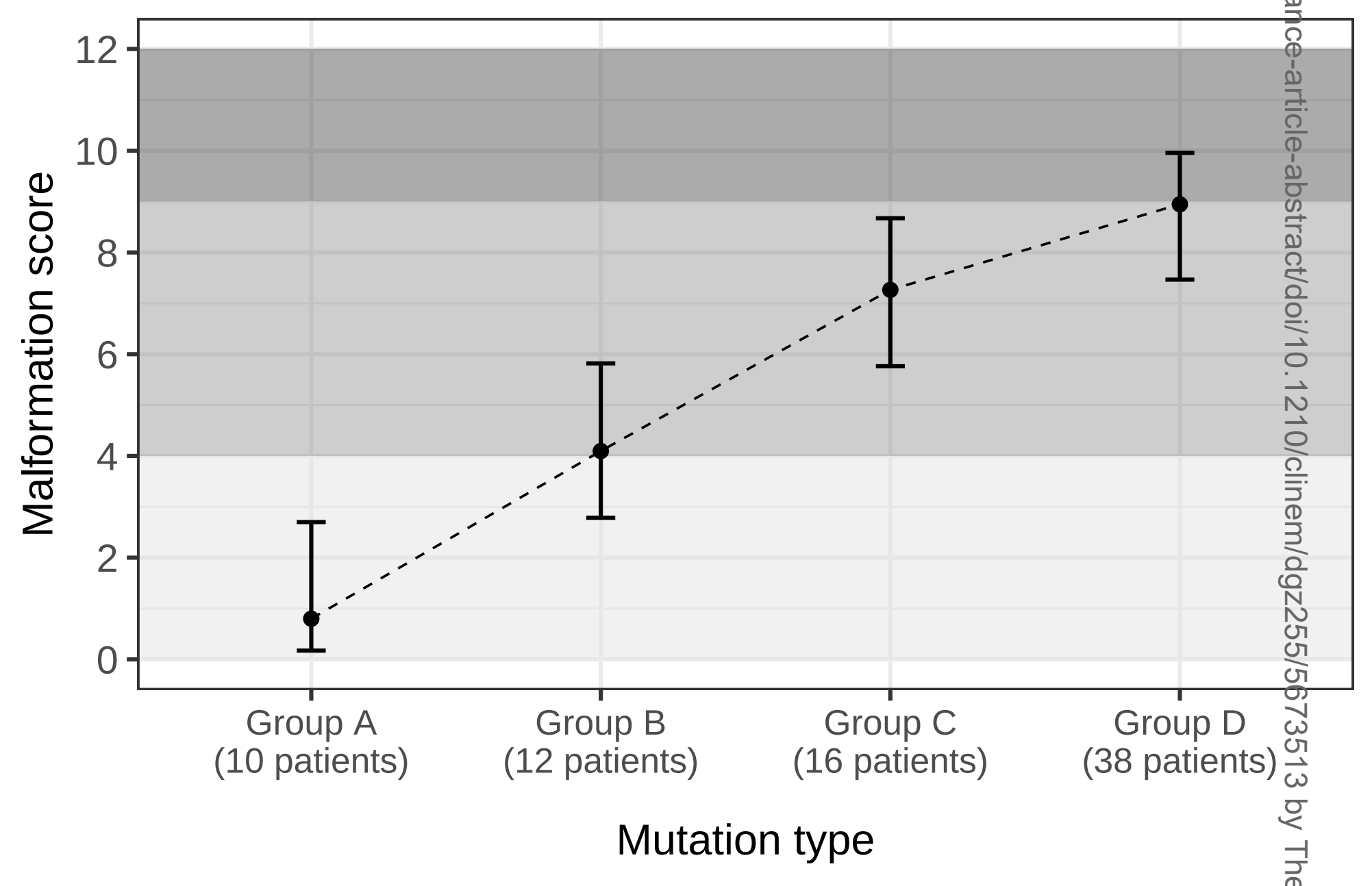











Malformation score
12
10
8
6
4
2
0
Group A
(10 patients)
Group B
(12 patients)
Group C
(16 patients)
Group D
(38 patients)
Mutation type