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Male sex, the levels of prolactin, presence of hypopituitarism and suprasellar extension are predictors of prolactinomas resistance to medical therapy

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Endocrinologia

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Male sex, the levels of prolactin, presence of hypopituitarism and suprasellar extension are predictors of prolactinomas resistance to medical therapy

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Faculdade de Medicina da Universidade do Porto, 24/03/2023

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DEDICATÓRIA

Dedico este trabalho aos meus pais e ao meu irmão, a minha base para tudo na vida e sem o apoio dos quais nada seria possível. Que bom ter a certeza de que para onde quer que vá, vos trago comigo no coração.

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Male sex, the levels of prolactin, presence of hypopituitarism and suprasellar extension are predictors of prolactinomas resistance to medical therapy

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Abstract

Introduction - International guidelines recommend dopamine agonists as first-line treatment strategy for prolactinomas. Dopaminergic agonists allow the normalization of prolactin levels and the reduction of the tumor dimension. Despite its high efficacy, some prolactinomas are resistant to this therapy. Therefore, this retrospective study aims to identify differences between resistant and responder prolactinomas, to better identify them in clinical practice. Methods - Clinical, laboratory, and imaging characteristics of a cohort of 122 prolactinomas, followed at Centro Hospitalar Universitário de São João, were analyzed. Resistance was defined as the use of cabergoline in doses superior to 3 mg/week without lowering prolactin levels or tumor size. Results are presented as mean ± standard deviation and compared as appropriated, namely using a univariate and multivariate analysis. Results - We considered 114 patients as responders (23.7% male; age 21.5 ± 11.0 years; mean prolactin levels 147.0 ng/mL; 2.8% without visible lesion, 52.8% micro and 44.4% macroprolactinomas; suprasellar extension in 28.2%; infrasellar extension in 28.0% and parasellar extension in 30.1%) and 8 resistant (62.5% male; age 16.8 ± 12.9 years; mean prolactin levels 2430.0 ng/mL; all of them were macroprolactinomas and 87.5% presented extension in at least one of the 3 considered dimensions). Responders used medium doses of 6.6 ± 6.3 mg/day of bromocriptine and 1.1 ± 0.6 mg/week of cabergoline, while resistants used 16.3 ± 11.1 mg/day of bromocriptine and 5.4 ± 4.3 mg/week of cabergoline. One resistant patient was treated with temozolomide. All resistant had hypogonadism and presented more pituitary hypofunctions than responders. Conclusion - Male sex, higher prolactin levels, hypopituitarism and suprasellar extension are predictors of prolactinomas resistance to dopaminergic agents.

Keywords: Prolactinoma; Dopamine Agonists; Dopaminergic Receptors; Pitutary Adenomas; Resistance.

Introduction

Prolactinomas are the most common hormone-secreting pituitary adenomas, accounting for approximately 50% of them, and with an estimated prevalence of 50 per 100,000 people [1]. They also represent the most frequent cause of pathological hyperprolactinemia although different other causes should be excluded in the differential diagnosis [2]. They affect more frequently women in the third to fifth decades of age, in whom they are commonly microprolactinomas, while older women and men more frequently present macroprolactinomas [3-5].

Clinical features of prolactinomas are characterized by signs and symptoms of hyperprolactinemia such as galactorrhoea, hypogonadism and infertility; In women, there are also described menstrual disorders, such as oligomenorrhea and amenorrhea, and reduced bone density due to the estrogens deficiency [3]; On the other hand, men are commonly affected with reduced libido and erectile dysfunction, which may be more insidious traits that lead to a later diagnosis [6]. Patients may present visual field loss, headache or cranial nerve palsy as well, due to direct adenoma mass effect [3].

In addition, affection of other pituitary hormonal lines has also been described [7]. The gonadotrophic line is the most affected (73-86%), due to the double effect of the prolactinoma in this axis: by the mass effect on the gonadotrophic cells, and by the prolactin (PRL) suppressor effect on hypothalamic GnRH secretion. Less frequently, hypothyroidism, hypocortisolism and growth hormone (GH) deficiency are also observed. Besides that, more than one hormone deficit can be present simultaneously, causing hypopituitarism.

Altogether, the main goals of the prolactinomas treatment are to normalize PRL levels, reduce the tumor size, reverse symptoms of hyperprolactinemia and restore gonadal function. Current international guidelines, including Endocrine Society [8], recommend medical therapy with dopamine agonists (DA) [1, 9], such as cabergoline and bromocriptine, as first-line treatment, since they are more effective than surgery. Both drugs are well documented at improving the mentioned markers and they are effective and usually well tolerated.

It should be noted that, despite the high efficacy of DA, 10-15% of prolactinomas do not respond satisfactorily to this therapy, leading to treatment failure and disease progression. [10]. DA pharmacology resistance is not consensual and different definitions have been proposed. No prolactin normalization, no prolactin normalization with bromocriptine doses of 15 to 30 mg/day for at least 3 months, no prolactin normalization after maximal tolerated dose of DA agents, no prolactin normalization with cabergoline doses of 3,5 mg/week, no prolactin normalization plus tumoral mass reduction of at least 30 or 50% were proposed definitions. [7, 8, 11]. DA resistance can occur due to several mechanisms. One of the most common ones is the downregulation or desensitization of dopamine receptors in prolactinoma cells. Therefore, exposure to DAs can lead to less response from the dopamine receptors to the drugs, which causes reduced inhibition of prolactin secretion. In some cases, genetic mutations in the dopamine receptor genes may also contribute to DA resistance.

For patients who don´t achieve desired results on standard doses of DA, it is recommended to increase to maximally tolerable doses [8]. In some cases, switching to a

different class of DA can also prove to be effective.

Shall medical therapy prove to be unsuccessful, neurosurgery through a transsphenoidal approach is universally considered second-line treatment [1, 12]. For those in whom surgery fails, radiation therapy or experimental targeted therapies should be considered.

To sum up, prolactinoma is a prevalent tumor [1], with a significant impact on patients' quality of life, particularly when improperly managed due to multiple causes, such as resistance to first-line therapy. Therefore, in this retrospective study, we aim to better identify resistant cases and their main hallmarks, as well as evaluate the most relevant differences between DA therapy responsive and resistant patients, both clinically and in the complementary study.

Materials and Methods

Patient population

We developed an observational retrospective study on patients who were diagnosed with prolactinomas at Centro Hospitalar Universitário de São João (CHUSJ) (Porto, Portugal). Clinical, laboratory and demographic data were reviewed from medical records, from 2000 to 2022. The study was approved by the Ethics Committee.

Patients were eligible for inclusion in the study if they were over 18 years of age and had a diagnosis of prolactinoma and they were excluded if there was no information about the date and prolactin level at diagnosis.

Clinical signs

During the patients' follow-up, clinical characteristics, gender, age at diagnosis, PRL levels at diagnosis, prolactinoma dimensions (microprolactinoma/macroprolactinoma), invasiveness (suprasellar, parasellar and infrasselar extension) and other endocrinology abnormalities (hypogonadism, hypothyroidism, hypocortisolism, GH deficiency, hypopituitarism) were collected.

Diagnosis timing was considered as the day when hyperprolactinemia was detected by blood analysis and/or pituitary adenoma was detected on Magnetic Resonance Imaging (MRI).

Based on the maximum tumor diameter, prolactinomas were defined as microprolactinomas if ≤10 mm and macroprolactinomas if >10mm. Prolactinoma invasiveness was determined using Knosp classification or reported invasion in neuroimaging.

Clinical response to therapy was evaluated with the following data: DA therapy chosen (bromocriptine [mg/day] and/or cabergoline [mg/week]) and alternative therapy needed (surgery, radiosurgery, tamoxifen, raloxifene, temozolomide).

DA resistance was defined as the use of cabergoline in doses superior to 3mg/ week or bromocriptine higher than 20mg/day without a response at lowering PRL levels or tumor size.

Statistical Analysis

Data collection and statistical analysis were performed using IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA). Results were compared as appropriated, namely using linear regression with univariate and multivariate analysis. Normally distributed continuous variables were represented as mean ± standard deviation (SD) and categorical variables were reported as absolute and relative frequencies (%). Comparisons between groups were performed with the Mann-Whitney test. Comparisons over time were performed with the Wilcoxon test. Spearman's correlation coefficient was used to assess correlations. A p-value of 0.05 or less was considered statistically significant.

Results

General characteristics of patients

A total of 122 patients with prolactinomas were included in this study. From those, we identified 114 patients that were responders to DA therapy and 8 resistant prolactinomas. Their general characteristics are represented in table 1.

At the time of diagnosis, among the total of 114 responder patients, there were 27 men (23.7%) and 87 women (76.3%), while in the group of resistant patients, 5 were men (62.5%) and 3 were women (37.5%) ($p = 0.016$).

However, regarding the age of diagnosis, it was not reported any significant difference between the appearance of responders and resistant prolactinomas (mean age 21.5± 11.0 vs. 16.8 ± 12.9 years, $p = 0.25$).

Prolactinomas dimension in the responder group was divided by 3 non-identifiable image in neuroimaging (2.8%), 57 micro- (52.8%), and 48 macro-prolactinomas (44.4%), whereas the 8 resistant patients presented only macroprolactinomas (100%) ($p < 0.01$).

Resistant prolactinomas globally had a higher percentage of supra-, infra- and para-sellar extension reported in MRI examination (87.5% resistant reported invasion and the responders had 28.2% of suprasellar, 28.0% of infrasselar and 30.1% of parasellar invasion; $p < 001$).

Initial PRL levels in responder prolactinomas were significantly lower than those in resistant patients (147.0 vs 2430.0 ng/mL, $p < 0.001$).

Therapeutic Characteristics

Among the 114 patients that responded to DA therapy, 56 were treated with bromocriptine (62.2%) and 34 with cabergoline (37.8%) and none of them needed to use the 2 pharmacological weapons at the same time. Meanwhile, 4 resistant patients used bromocriptine (50.0%), 2 of them cabergoline (25.0%) and the other 2 used both DA (25.0%). (Fig. 1).

The doses of DA used in this patient's treatment are exposed in table 2.

Bromocriptine was used at the mean dose of 6.6 ± 6.3 mg/day in the responder's group, whereas it needed a mean of 16.3 ± 11.1 mg/day in the resistant one ($p < 0.01$).

When cabergoline was used, responders, on average used 1.1 ± 0.6 mg/week, and resistant patients had to reach a mean of 5.4 ± 4.3 mg/week ($p = 0.007$); there was also 1 patient with a resistant prolactinoma that was treated with temozolomide (12.5%; $p < 0.01$).

In addition to the use of DA, 2 responders, and 6 resistant patients had to undergo surgery (3.0% vs 75.0% ; $p < 0.001$), and radiosurgery was only required for 3 patients that belonged to the resistant group (0.0% vs 37.5% ; $p < 0.01$).

Clinical features of patients

Regarding the clinical background of the included patients, hypopituitarism was described in 2 responders and 3 resistant (2.4% vs 37.5% ; $p < 0.01$); whereas 22 responders had hypogonadism, while all of the resistant described that clinical features (25.9% vs 100.0% ; $p < 0.01$); hypothyroidism was present in 2 responders and 3 resistant (3.6% vs 37.5% ; $p < 0.05$); 3 responders and 4 resistant had hypocortisolism (3.6% vs 50.0% ; $p < 0.01$); Deficiency in GH line was reported in 2 patients that responded to the therapeutics and in only 1 of the resistants' (12.5% ; $p = 0.14$). The relative frequency of these findings is described in Graph 2.

We were able to evaluate the impact of different predictors in resistance, through linear regression with a univariate and multivariate analysis of the resistant patient's characteristics. These results are represented in table 3.

Discussion

As it stands, DAs are the first-line treatment for prolactinomas, and work by binding to dopamine receptors in the prolactinoma cells, which inhibits the over-secretion of prolactin, reducing the symptoms caused by the high levels of prolactin and reducing the mass effect of the tumor. However, some individuals had tumors resistant to these drugs, which makes their treatment more difficult, and it is often necessary to use second-line treatments, which tend to be less effective and more invasive. Therefore, DA resistance is a challenging clinical problem, and early detection and prompt management of this limitation can help prevent disease progression and improve patient outcomes.

It is important to define the most susceptible population to this problem. The present study enrolled 114 patients that had good results with DA therapy and 8 patients that showed a refractory response and was able to study their main characteristics in real-life conditions.

This allowed us to prove that resistant prolactinomas tend to appear more frequently in the male gender than in the female ($p = 0.016$), which can be connected to the latest diagnosis that happens more frequently in men, due to the less specific symptoms, when compared to women. The Odds ratio of 5.37 (1.20 - 23.95 ; 0.028) gives the idea that the male gender is a strong predictor of resistant prolactinomas.

A literature review reported that refractory prolactinomas appear more frequently in elder people, particularly when talking about the female gender, however, our study result concerning the age of diagnosis did not reveal a significant difference regarding this topic, proving that the age is not a determinant factor when talking about the possibility to predict when a tumor is going to be resistant to the standard therapy with DA (21.5± 11.0 vs. 16.8 ± 12.9 years, $p = 0.25$). The OR of 0.98 (0.92 – 1.03) supports the lack of influence of this predictor in refractoriness.

Prolactin dosage at diagnosis was defined in our study as a contributing factor to the development of a resistant disease (147.0 vs 2430.0 ng/ml, $p < 0.001$). This higher PRL levels can be explained by both a late diagnosis or the occurrence of a more aggressive tumor, with greater rates of PRL production at presentation. Multivariate analysis on this topic shows an OR of 1.74 (0.97 – 3.12), demonstrating the effect of this single feature, when compared to others.

As described in previous studies, tumor dimension gains a relevant connection with the resistance of prolactinomas. All the resistant tumors included in this study were over 10 mm, proving once more this association between macroprolactinomas and their mechanisms leading to resistance (100% vs 44.4%; $p < 0.01$). Even more so, the tumor invasion for the cavernous sinus, the diaphragm, or the lower limits of the sella turcica was also correlated with the identification of resistant cases. 87.5% of the included refractory cases presented these invasive features in at least one of the three considered dimensions (supra-, infra- and parasellar). When compared to the responders, that showed lower rates of invasion (28.2%, 28.0%, and 30. 1%, respectively), we can find a notorious disparity. Although all of them seemed to have higher incidence in resistant cases, the multivariate analysis only showed a relevant impact regarding the suprasellar invasion. Therefore, suprasellar invasion represents a stronger predictor of resistance when talking about prolactinomas [OR = 5.14 (1.04 – 220.67)].

Standard treatment for prolactinomas is worldwide accepted as DA therapy, with the goal to reduce the production of prolactin, alleviate the symptoms and shrink the tumor. All the included patients in this study started their therapy strategy with medical therapy, with one of the two considered DAs, cabergoline or bromocriptine. The average dosage used revealed that suppressing PRL to normal or close to normal levels in responder patients was achieved with a lower amount of DA (6.6 ± 6.3 mg/day and 1.1 ± 0. ,6 mg/week of bromocriptine and cabergoline, respectively). Most refractory patients were not able to achieve satisfactory results concerning PRL levels or tumor size with standard DA therapy. Proof of this lack of responsiveness is the dose of DA that they had to go through during their treatment (16.3 ± 11.1 mg/day and 5.4 ± 4.3 mg/week of

bromocriptine and cabergoline, respectively). In addition, one of them was submitted to temozolomide as an alternative treatment to try to reach the wanted results.

Giraldi et al [13], a retrospective study with 26 resistant prolactinoma patients, reported that these tumors usually require a multi-modal treatment approach, consisting in increasing DA dose and, if not sufficient, surgery and radiotherapy. 75% of our resistant prolactinomas underwent transsphenoidal surgery and 37.5% of them had to get to the point of radiosurgery. On the other hand, only 2 of the responders needed surgery and none had to be submitted to radiosurgery. This considerable difference between the two groups reveals the low rate of control obtained with DA when talking about resistant cases.

Regarding clinical symptoms found in the included 122 patients, women often showed menstrual abnormalities such as amenorrhea and oligomenorrhea and men commonly appeared with libido deregulation and erectile dysfunction. Galactorrhea, gynecomastia, and headache were also very frequently reported symptoms. However, when talking about other pituitary hormone production, resistant prolactinomas showed a higher incidence of hypoproduction.

The growth and expansion of prolactinomas can induce compression of the neighboring cells in the pituitary gland, affecting other hormone production, which are interconnected and regulated by feedback mechanisms. Besides that, the actual rise in PRL can also cause these anomalies. Excess PRL can inhibit the release of GnRH, which stimulates FSH and LH production, interfere with the release of TSH, inhibit ACTH production, and affect GH release.

In this study, hypogonadism hypogonadotrophic was the most reported abnormality in both groups. Nevertheless, all 8 resistant showed this feature (100.0%), while less considerable rates of responders were reported (25.9%; $p < 0.01$), giving rise to the hypothesis that resistant prolactinomas have a significant impact in the pituitary gland.

Non-resistant cases had considerably lower scales of other hormone anomalies. Only 2.4-3.6% of them presented some irregularity connected to disruption of other hormone production other than sexual hormones. On the other hand, half of the resistant sample described hypocortisolism, 37.5% showed hypothyroidism and a smaller percentage of them reported abnormalities concerning GH line (12.5%). The affection of at least more than one hormone production (hypopituitarism), had a relevant significative difference between non- and responder groups, since 37.5% of the resistant and only 2.4% of responders had this clinical problem.

High rates of hypogonadism individually can be explained by the effect of PRL in GnRH release, which has a direct impact on sexual hormone production in the gonadal organs. However, the multivariate analysis concerning hypopituitarism shows that this clinical feature is influenced itself by the resistant characteristic of the tumor, which may result from the mass effect that the growing mass had in the pituitary gland [OR = 37.20 (1.98 – 699.04) in multivariate analysis; $p = 0.016$]. Based on this study's results, hypopituitarism appears to be a strong predictor when talking about the rate of resistance of these tumors.

One limitation of this study was related to the fact that this was a survey conducted in one individual hospital. Thus, our results may not represent wide characteristics of prolactinomas generally, because of cluster segregation of features related to geographic matters.

In conclusion, our retrospective study reviewed clinical data for 122 patients, comparing resistant and responder patients to DA therapy, and found that resistant prolactinomas are more associated with the male gender, have higher PRL levels, bigger dimensions, more supra-, lateral- and infra-sellar invasion, more pituitary hypofunction, and age is not a relevant predictor.

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Table 1. General characteristics of included patients.

Clinical features	Responders (n = 114)	Resistant (n = 8)	P value
Sex (n)			0.016
Male	27 (23.7%)	5 (62.5%)	
Female	87 (76.3%)	3 (37.5%)	
Mean age at diagnosis (years)	21.5 ± 11.0	16.8 ± 12.9	0.25
Prolactinoma dimension (n)			0.010
0	3 (2.8%)	0 (0.0%)	
Microprolactinoma	57 (52.8%)	0 (0.0%)	
Macroprolactinoma	48 (44.4%)	8 (100.0%)	
Extension (n)			
Suprasellar extension	29 (28.2%)	7 (87.5%)	<0.01
Infrasellar extension	28 (28.0%)	7 (87.5%)	<0.01
Parasellar extension	31 (30.1%)	7 (87.5%)	<0.01
PRL level at diagnosis (ng/mL)	147,0 [94.5 ; 470.0]	2430.0 [1928.5 ; 3139.0]	<0.01

Fig. 1. DA therapy. **A**. Responders. **B.** Resistant.

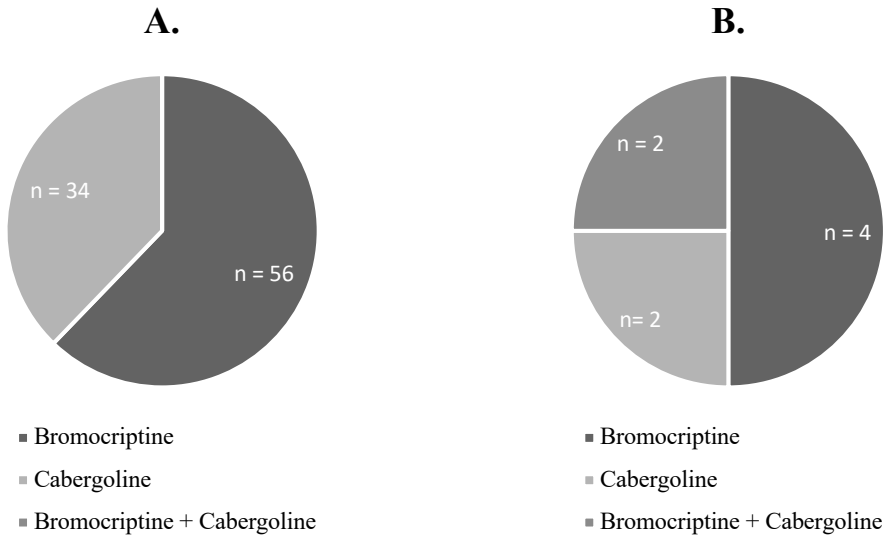

Table 2. Doses of DA used in responders and resistant patients.

Therapy	Responders (n = 114)	Resistant (n = 8)	P value
Pharmacological therapy			
Bromocriptine (mg/day)	6.6 ± 6.3	16.3 ± 11.1	0.07
Cabergoline (mg/week)	1.1 ± 0.6	5.4 ± 4.3	<0.01
Temozolomide	0 (0.0%)	1 (12.5%)	<0.01
Others			
Surgery	2 (3.0%)	6 (75.0%)	<0.01
Radiosurgery	0 (0. 0%)	3 (37.5%)	<0.01

Fig 2. Clinical pituitary features in responders and resistant patients.

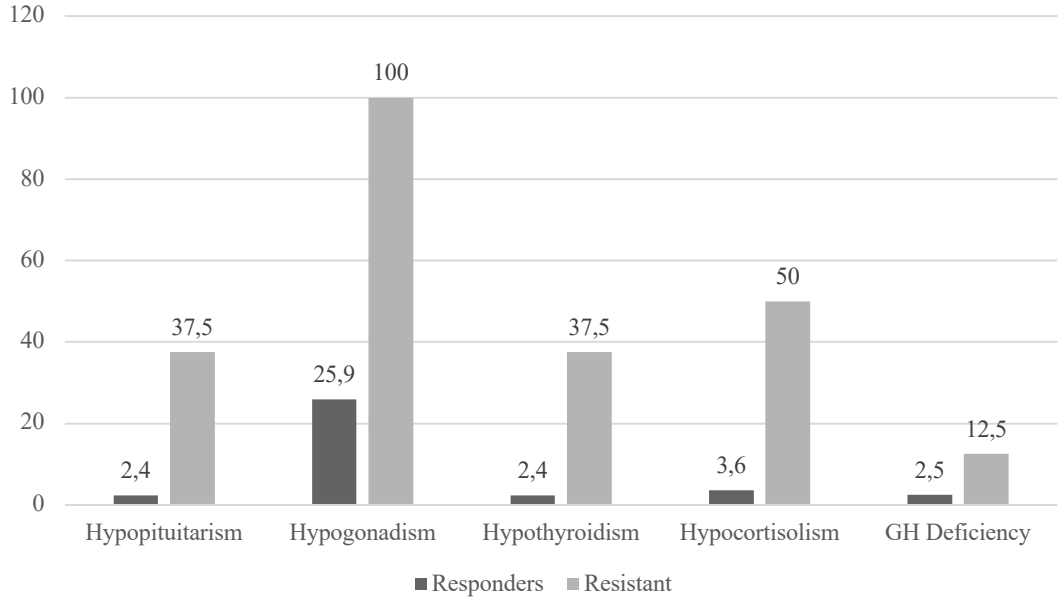

Table 3. Logistic regression of predictors of resistance to treatment.

A. Univariate analysis

	Odds ratio	*P value*
Male sex	5.37 (1.20 - 23.95)	0.028
Age at diagnosis	0.98 (0.92 - 1.03)	0.403
Prolactinoma dimension*	1.00 (1.00 - 1.00)	
Suprasellar extension	17.86 (2.10 - 151.64)	0.008
Infrasellar extension	18.00 (2.12 - 153.03)	0.008
Parasellar extension	16.26 (1.92 - 137.79)	0.011
PRL level at diagnosis	2.13 (1.34 - 3.39)	0.002
Hypopituitarism	24.30 (3.27 - 180.31)	0.002
Hypogonadism*	1.00 (1.00 - 1.00)	
Hypothyroidism	24.30 (3.27 - 180.31)	0.002
Hypocortisolism	27.00 (4.45 - 163.73)	0.000
GH deficiency	5.50 (0.44 - 68.50)	0.185

B. Multivariate analysis

	Odds ratio	*P value*
Male sex	3.10 (0.45 – 21.49)	0.253
PRL level at diagnosis	1.74 (0.97 – 3.12)	0.064
Hypopituitarism	37.20 (1.98 – 699.04)	0.016
Suprasellar extension	15.14 (1.04 – 220.67)	0.047

*Odds ratio of this predictor cannot be calculated because all of the resistant prolactinomas presented this characteristic.

ANEXOS

A) STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Guidelines.

B) Normas de publicação da revista: *Frontiers in Endocrinology*

A) REPORTING GUIDELINES - CHECKLIST

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	7
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	7
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	8
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	9
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	9-10
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	10
		(*d*) If applicable, explain how loss to follow-up was addressed	--
		(*e*) Describe any sensitivity analyses	10
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	--
		(c) Consider use of a flow diagram	--
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10,12
		(b) Indicate number of participants with missing data for each variable of interest	--
		(c) Summarise follow-up time (eg, average and total amount)	--
Outcome data	15*	Report numbers of outcome events or summary measures over time	10-12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12
		(*b*) Report category boundaries when continuous variables were categorized	--

		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	--
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	--

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

REPORTING GUIDELINES – EXAMPLES

1 - "Therefore, this retrospective study aims to identify differences between resistant and responder prolactinomas, to better identify them in clinical practice. (…) Clinical, laboratory, and imaging characteristics of a cohort of 122 prolactinomas, followed at Centro Hospitalar Universitário de São João, were analyzed (…) Male sex, higher prolactin levels, hypopituitarism and suprasellar extension are predictors of prolactinomas resistance to dopaminergic agents.**"**

2 - "Current international guidelines, including Endocrine Society, recommend medical therapy with dopamine agonists (DA), such as cabergoline and bromocriptine, as first-line treatment, since they are more effective than surgery. (…) It should be noted that, despite the high efficacy of DA, 10-15% of prolactinomas do not respond satisfactorily to this therapy, leading to treatment failure and disease progression."

3 - "Therefore, in this retrospective study, we aim to better identify resistant cases and their main hallmarks, as well as evaluate the most relevant differences between DA therapy responsive and resistant patients, both clinically and in the complementary study."

4 - "We developed an observational retrospective study on patients who were diagnosed with prolactinomas at Centro Hospitalar Universitário de São João (CHUSJ)"

5 - "at Centro Hospitalar Universitário de São João (CHUSJ) (Porto, Portugal). Clinical, laboratory and demographic data were reviewed from medical records, from 2000 to 2022."

6 - "Patients were eligible for inclusion in the study if they were over 18 years of age and had a diagnosis of prolactinoma and they were excluded if there was no information about the date and prolactin level at diagnosis."

7 - "During the patients' follow-up, clinical characteristics, gender, age at diagnosis, PRL levels at diagnosis, prolactinoma dimensions (microprolactinoma/macroprolactinoma), invasiveness (suprasellar, parasellar and infrasselar extension) and other endocrinology abnormalities (hypogonadism, hypothyroidism, hypocortisolism, GH deficiency, hypopituitarism) were collected. (…) Clinical response to therapy was evaluated with the following data: DA therapy chosen (bromocriptine [mg/day] and/or cabergoline [mg/week]) and alternative therapy needed (surgery, radiosurgery, tamoxifen, raloxifene, temozolomide). DA resistance was defined as the use of cabergoline in doses superior to 3mg/ week without a response at lowering PRL levels or tumor size."

8 - "Diagnosis timing was considered as the day when hyperprolactinemia was detected by blood analysis and/or pituitary adenoma was detected on Magnetic Resonance Imaging (MRI). Based on the maximum tumor diameter, prolactinomas were defined as microprolactinomas if ≤10 mm and macroprolactinomas if >10mm. Prolactinoma invasiveness was determined using Knosp classification or reported invasion in neuroimaging."

9 - "Patients were eligible for inclusion in the study if they were over 18 years of age and had a diagnosis of prolactinoma and they were excluded if there was no information about the date and prolactin level at diagnosis."

10 - "We developed an observational retrospective study on patients who were diagnosed with prolactinomas at Centro Hospitalar Universitário de São João (CHUSJ) (Porto, Portugal). Clinical, laboratory and demographic data were reviewed from medical records, from 2000 to 2022."

11 - "Normally distributed continuous variables were represented as mean ± standard deviation (SD)".

12 - "Data collection and statistical analysis were performed using IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA). Results were compared as appropriated, namely using univariate and multivariate analysis. Normally distributed continuous variables were represented as mean ± standard deviation (SD) and categorical variables were reported as absolute and relative frequencies (%). Comparisons between groups were performed with the Mann-Whitney test. Comparisons over time were performed with the Wilcoxon test. Spearman's correlation coefficient was used to assess correlations. A p-value of 0.05 or less was considered statistically significant."

13 - "A total of 122 patients with prolactinomas were included in this study. From those, we identified 114 patients that were responders to DA therapy and 8 resistant prolactinomas."

14 - "At the time of diagnosis, among the total of 114 responder patients, there were 27 men (23.7%) and 87 women (76.3%), while in the group of resistant patients, 5 were men (62.5%) and 3 were women (37.5%) ($p = 0.016$). (…) Regarding the clinical background of the included patients, panhypopituitarism was described in 2 responders and 3 resistant (2.4% vs 37.5% ; $p < 0.01$); whereas 22 responders had hypogonadism, while all of the resistant described that clinical feature (25.9% vs 100.0% ; $p < 0.01$); hypothyroidism was present in 2 responders and 3 resistant (3.6% vs 37.5% ; $p < 0.05$); 3 responders and 4 resistant had hypocortisolism (3.6% vs 50.0% ; $p < 0.01$); Deficiency in GH line was reported in 2 patients that responded to the therapeutics and in only 1 of the resistants' (12.5% ; $p = 0.14$)."

15 + 16 - "there were 27 men (23.7%) and 87 women (76.3%), while in the group of resistant patients, 5 were men (62.5%) and 3 were women (37.5%) ($p = 0.016$). However, regarding the age of diagnosis, it was not reported any significant difference between the appearance of responders and resistant prolactinomas (mean age 21.5± 11.0 vs. 16.8 ± 12.9 years, $p = 0.,25$). Prolactinomas dimension in the responder group was divided by 3 non-measurable in neuroimaging (2,8%), 57 micro- (52.8%), and 48 macro-prolactinomas (44.4%), whereas the 8 resistant patients presented only macroprolactinomas (100%) ($p < 0.01$). Resistant prolactinomas globally had a higher percentage of supra-, infra- and para-sellar extension reported in MRI examination (87.5% resistant reporteded invasion and the responders had 28.2% of suprasellar, 28.0% of infrasselar and 30.1% of parasellar invasion; $p < 001$). Initial PRL levels in responder prolactinomas were significantly lower than those in resistant patients (147.0 vs 2430.0 ng/ml, $p < 0.001$). (…) Bromocriptine was used at the mean dose of 6.6 ± 6.3 mg/day in

the responder's group, whereas it needed a mean of 16.3 ± 11.1 mg/day in the resistant one ($p < 0.01$). When cabergoline was used, responders, on average used 1.1 ± 0.6 mg/week, and resistant patients had to reach a mean of 5.4 ± 4.3 mg/week ($p = 0.007$); there was also 1 patient with a resistant prolactinoma that had to resort to temozolomide (12.5%; $p < 0.01$). (…) panhypopituitarism was described in 2 responders and 3 resistant (2.4% vs 37.5% ; $p < 0.01$); whereas 22 responders had hypogonadism, while all of the resistant described that clinical feature (25.9% vs 100.0% ; $p < 0.01$); hypothyroidism was present in 2 responders and 3 resistant (3.6% vs 37.5% ; $p < 0.05$); 3 responders and 4 resistant had hypocortisolism (3.6% vs 50.0% ; $p < 0.01$); Deficiency in GH line was reported in 2 patients that responded to the therapeutics and in only 1 of the resistants' (12.5% ; $p = 0.14$)."

17 - "We were able to evaluate the impact of different predictors in resistance, through a univariate and multivariate analysis of the resistant patient's characteristics. These results are represented in table 3."

18 - "prolactinomas are more associated with the male gender, have higher PRL levels, bigger dimensions, more supra-, lateral- and infra-sellar invasion, more pituitary hypofunction, and age is not a relevant predictor."

19 - "One limitation of this study was related to the fact that this was a survey conducted in one individual hospital. Thus, our results may not represent wide characteristics of prolactinomas generally, because of cluster segregation of features related to geographic matters."

20 - "This allowed us to prove that resistant prolactinomas tend to appear more frequently in the male gender than in the female ($p = 0.016$), which can be connected to the latest diagnosis that happens more frequently in men, due to the less specific symptoms, when compared to women. (…) age is not a determinant factor when talking about the possibility to predict when a tumor is going to be resistant to the standard therapy with DA (21.5± 11.0 vs. 16.8 ± 12.9 years, $p = 0.25$). (…) Prolactin dosage at diagnosis was defined in our study as a contributing factor to the development of a resistant disease (147.0 vs 2430.0 ng/ml, $p < 0.001$) (…) suprasellar invasion represents a stronger predictor of resistance when talking about prolactinomas (…) the multivariate analysis concerning hypopituitarism shows that this clinical feature is influenced itself by the resistant characteristic of the tumor, which may result from the mass effect that the growing mass had in the pituitary stalk [OR = 37.20 (1.98 – 699.04) in multivariate analysis ; $p = 0.016$]."

21 - "our results may not represent wide characteristics of prolactinomas generally, because of cluster segregation of features related to geographic matters."

22 - not applicable because no mention to funders was made.

B) NORMAS DE PUBLICAÇÃO DA REVISTA

Author guidelines - *Frontiers in Endocrinology*

A. General standards

1. Article type

Frontiers requires authors to select the appropriate article type for their manuscript and to comply with the article type descriptions defined in the journal's 'Article types' page, which can be found under the 'About journal' menu in 'For authors' on every Frontiers journal page. Please pay close attention to the word count limits.

2. Templates

If working with Word please use our Word templates. If you wish to submit your article as LaTeX, we recommend our LaTeX templates.

For LaTeX files, please ensure all relevant manuscript files are uploaded: .tex file, PDF, and .bib file (if the bibliography is not already included in the .tex file).

During the interactive review, authors are encouraged to upload versions using track changes. Editors and reviewers can only download the PDF file of the submitted manuscript.

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We encourage you to closely follow the article word count lengths given in the 'Article types' page of the journals. The manuscript length includes only the main body of the text, footnotes, and all citations within it, and excludes the abstract, section titles, figure and table captions, funding statement, acknowledgments, and references in the bibliography. Please indicate the number of words and the number of figures and tables included in your manuscript on the first page.

4. Language editing

Frontiers requires manuscripts submitted to meet international English language standards to be considered for publication.

For authors who would like their manuscript to receive language editing or proofreading to improve the clarity of the manuscript and help highlight their research, we recommend the language-editing services provided by the following external partners.

Note that sending your manuscript for language editing does not imply or guarantee that it will be accepted for publication by a Frontiers journal. Editorial

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Frontiers recommends the Charlesworth Group's author services, who has a long-standing track record in language editing and proofreading. This is a third-party service for which Frontiers authors will receive a 10% discount by visiting the following link: www.cwauthors.com/frontiers.

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The default language style at Frontiers is American English. If you prefer your article to be formatted in British English, please specify this on the first page of your manuscript. For any questions regarding style, Frontiers recommends authors to consult the Chicago Manual of Style.

6. **Search engine optimization (SEO)**

There are a few simple ways to maximize your article's discoverability and search results.

- Include a few of your article's keywords in the title of the article
- Do not use long article titles
- Pick 5-8 keywords using a mix of generic and more specific terms on the article subject(s)
- Use the maximum amount of keywords in the first two sentences of the abstract
- Use some of the keywords in level 1 headings

B. CrossMark policy

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Clicking on the CrossMark logo will tell you the current status of a document and may also give you additional publication record information about the document.

a) Title

The title should be concise, omitting terms that are implicit and, where possible, be a statement of the main result or conclusion presented in the manuscript. Abbreviations should be avoided within the title.

Witty or creative titles are welcome, but only if relevant and within measure. Consider if a title meant to be thought-provoking might be misinterpreted as offensive or alarming. In extreme cases, the editorial office may veto a title and propose an alternative.

Authors should avoid:

- titles that are a mere question without giving the answer
- unambitious titles, for example starting with 'Towards,' 'A description of,' 'A characterization of' or 'Preliminary study on'
- vague titles, for example starting with 'Role of', 'Link between', or 'Effect of' that do not specify the role, link, or effect
- including terms that are out of place, for example the taxonomic affiliation apart from species name.

For Corrigenda, General Commentaries, and Editorials, the title of your manuscript should have the following format.

- 'Corrigendum: [Title of original article]'
- General Commentaries:
  'Commentary: [Title of original article]'
  'Response: Commentary: [Title of original article]'
- 'Editorial: [Title of Research Topic]'

2. **Authors and affiliations**

All names are listed together and separated by commas. Provide exact and correct author names as these will be indexed in official archives. Affiliations

should be keyed to the author's name with superscript numbers and be listed as follows:

- Laboratory, Institute, Department, Organization, City, State abbreviation (only for United States, Canada, and Australia), and Country (without detailed address information such as city zip codes or street names).

Example: Max Maximus1
1 Department of Excellence, International University of Science, New York, NY, United States.

a) Correspondence

The corresponding author(s) should be marked with an asterisk in the author list. Provide the exact contact email address of the corresponding author(s) in a separate section.

Example: Max Maximus*
maximus@iuscience.edu

If any authors wish to include a change of address, list the present address(es) below the correspondence details using a unique superscript symbol keyed to the author(s) in the author list.

b) Equal contributions

The authors who have contributed equally should be marked with a symbol (†) in the author list of the doc/latex and pdf files of the manuscript uploaded at submission.

Please use the appropriate standard statement(s) to indicate equal contributions:

- **Equal contribution:** These authors contributed equally to this work
- **First authorship:** These authors share first authorship
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- **Last authorship:** These authors share last authorship
- **Equal contribution and first authorship:** These authors contributed equally to this work and share first authorship
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Example: Max Maximus 1†, John Smith2† and Barbara Smith1
†These authors contributed equally to this work and share first authorship

3. **Consortium/group and collaborative authors**

Consortium/group authorship should be listed in the manuscript with the other author(s).

In cases where authorship is retained by the consortium/group, the consortium/group should be listed as an author separated by a comma or 'and'. The consortium/group name will appear in the author list, in the citation, and in the copyright. If provided, the consortium/group members will be listed in a separate section at the end of the article.

For the collaborators of the consortium/group to be indexed in PubMed, they do not have to be inserted in the Frontiers submission system individually. However, in the manuscript itself, provide a section with the name of the consortium/group as the heading followed by the list of collaborators, so they can be tagged accordingly and indexed properly.

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In cases where work is presented by the author(s) on behalf of a consortium/group, it should be included in the author list separated with the wording 'for' or 'on behalf of.' The consortium/group will not retain authorship and will only appear in the author list.

Example: John Smith and Barbara Smith on behalf of The Collaborative Working Group.

4. **Abstract**

As a primary goal, the abstract should make the general significance and conceptual advance of the work clearly accessible to a broad readership. The abstract should be no longer than a single paragraph and should be structured, for example, according to the IMRAD format. For the specific structure of the abstract, authors should follow the requirements of the article type or journal to which they're submitting. Minimize the use of abbreviations and do not cite references, figures or tables.

For clinical trial articles, please include the unique identifier and the URL of the publicly-accessible website on which the trial is registered.

5. **Keywords**

All article types require a minimum of five and a maximum of eight keywords.

6. **Text**

The entire document should be single-spaced and must contain page and line numbers in order to facilitate the review process. The manuscript should be written using either Word or LaTeX. See above for templates.

7. **Nomenclature**

The use of abbreviations should be kept to a minimum. Non-standard abbreviations should be avoided unless they appear at least four times, and must be defined upon first use in the main text. Consider also giving a list of non-standard abbreviations at the end, immediately before the acknowledgments.

Equations should be inserted in editable format from the equation editor.

Italicize gene symbols and use the approved gene nomenclature where it is available. For human genes, please refer to the HUGO Gene Nomenclature Committee (HGNC). New symbols for human genes should be submitted to the HGNC here. Common alternative gene aliases may also be reported, but should not be used alone in place of the HGNC symbol. Nomenclature committees for other species are listed here. Protein products are not italicized.

We encourage the use of Standard International Units in all manuscripts.

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Astronomical objects should be referred to using the nomenclature given by the International Astronomical Union (IAU) provided here.

Life Science Identifiers (LSIDs) for ZOOBANK registered names or nomenclatural acts should be listed in the manuscript before the keywords. An LSID is represented as a uniform resource name (URN) with the following format: urn:lsid:<Authority>:<Namespace>:<ObjectID>[:<Version>]

For more information on LSIDs please see the 'Code' section of our policies and publication ethics.

8. **Sections**

The manuscript is organized by headings and subheadings. The section headings should be those appropriate for your field and the research itself. You may insert up to 5 heading levels into your manuscript (i.e.,: 3.2.2.1.2 Heading Title).

For Original Research articles, it is recommended to organize your manuscript in the following sections or their equivalents for your field.

Introduction
Succinct, with no subheadings.

Materials and methods
This section may be divided by subheadings and should contain sufficient detail so that when read in conjunction with cited references, all procedures can be repeated. For experiments reporting results on animal or human subject research, an ethics approval statement should be included in this section (for further information, see the 'Bioethics' section of our policies and publication ethics.)

Results
This section may be divided by subheadings. Footnotes should not be used and must be transferred to the main text.

Discussion
This section may be divided by subheadings. Discussions should cover the key findings of the study: discuss any prior research related to the subject to place the novelty of the discovery in the appropriate context, discuss the potential shortcomings and limitations on their interpretations, discuss their integration into the current understanding of the problem and how this advances the current views, speculate on the future direction of the research, and freely postulate theories that could be tested in the future.

For further information, please check the descriptions defined in the journal's 'Article types' page, in the 'For authors' menu on every journal page.

9. **Acknowledgements**

This is a short text to acknowledge the contributions of specific colleagues, institutions, or agencies that aided the efforts of the authors. Should the content of the manuscript have previously appeared online, such as in a thesis or preprint, this should be mentioned here, in addition to listing the source within the reference list.

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When you submit your manuscript, you will be required to briefly summarize in 200 words your manuscript's contribution to, and position in, the existing literature in your field. This should be written avoiding any technical language or non-standard acronyms. The aim should be to convey the meaning and importance of this research to a non-expert.

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Example statement on: Markram K and Markram H (2010) The Intense World Theory – a unifying theory of the neurobiology of autism. Front. Hum. Neurosci. 4:224. doi: 10.3389/fnhum.2010.00224

Autism spectrum disorders are a group of neurodevelopmental disorders that affect up to 1 in 100 individuals. People with autism display an array of symptoms encompassing emotional processing, sociability, perception and memory, and present as uniquely as the individual. No theory has suggested a single underlying neuropathology to account for these diverse symptoms. The Intense World Theory, proposed here, describes a unifying pathology producing the wide spectrum of manifestations observed in autists. This theory focuses on the neocortex, fundamental for higher cognitive functions, and the limbic system, key for processing emotions and social signals. Drawing on discoveries in animal models and neuroimaging studies in individuals with autism, we propose how a combination of genetics, toxin exposure and/or environmental stress could produce hyper-reactivity and hyper-plasticity in the microcircuits involved with perception, attention, memory and emotionality. These hyper-functioning circuits will eventually come to dominate their neighbors, leading to hyper-sensitivity to incoming stimuli, over-specialization in tasks and a hyper-preference syndrome. We make the case that this theory of enhanced brain function in autism explains many of the varied past results and resolves conflicting findings and views and makes some testable experimental predictions.

C. Figure and table guidelines

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All figures, tables, and images will be published under a Creative Commons CC-BY license, and permission must be obtained for use of copyrighted material from other sources (including re-published/adapted/modified/partial figures and images from the internet). It is the responsibility of the authors to acquire the licenses, follow any citation instructions requested by third-party rights holders, and cover any supplementary charges.

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Frontiers requires figures to be submitted individually, in the same order as they are referred to in the manuscript; the figures will then be automatically embedded at the end of the submitted manuscript. Kindly ensure that each figure is mentioned in the text and in numerical order.

For figures with more than one panel, panels should be clearly indicated using labels (A), (B), (C), (D), etc. However, do not embed the part labels over any part of the image, these labels will be replaced during typesetting according to Frontiers' journal style. For graphs, there must be a self-explanatory label (including units) along each axis.

For LaTeX files, figures should be included in the provided PDF. In case of acceptance, our production office might require high-resolution files of the figures included in the manuscript in EPS, JPEG or TIF/TIFF format.

To upload more than one figure at a time, save the figures (labeled in order of appearance in the manuscript) in a zip file and upload them as 'Supplementary Material Presentation.'

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Figures should be prepared with the PDF layout in mind. Individual figures should not be longer than one page and with a width that corresponds to 1 column (85 mm) or 2 columns (180 mm).

All images must have a resolution of 300 dpi at final size. Check the resolution of your figure by enlarging it to 150%. If the image appears blurry, jagged, or has a stair-stepped effect, the resolution is too low.

The text should be legible and of high quality. The smallest visible text should be no less than eight points in height when viewed at actual size.

Solid lines should not be broken up. Any lines in the graphic should be no smaller than two points wide.

Please note that saving a figure directly as an image file (JPEG, TIF) can greatly affect the resolution of your image. To avoid this, one option is to export the file as PDF, then convert into TIFF or EPS using a graphics software.

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The following formats are accepted: TIF/TIFF (.tif/.tiff), JPEG (.jpg), and EPS (.eps) (upon acceptance). Images must be submitted in the color mode RGB.

d) Chemical structures

Chemical structures should be prepared using ChemDraw or a similar program. If working with ChemDraw please use our ChemDraw template. If working with another program please follow the guidelines below.

- Drawing settings: chain angle, 120° bond spacing, 18% width; fixed length, 14.4 pt; bold width, 2.0 pt; line width, 0.6 pt; margin width, 1.6 pt; hash spacing, 2.5 pt. Scale 100% Atom Label settings: font, Arial; size, 8 pt
- Assign all chemical compounds a bold, Arabic numeral in the order in which the compounds are presented in the manuscript text.

3. Table requirements and style guidelines

Tables should be inserted at the end of the manuscript in an editable format. If you use a word processor, build your table in Word. If you use a LaTeX processor, build your table in LaTeX. An empty line should be left before and after the table.

Table captions must be placed immediately before the table. Captions should be preceded by the appropriate label, for example 'Table 1.' Please use only a single paragraph for the caption.

Ensure that each table is mentioned in the text and in numerical order.

Large tables covering several pages cannot be included in the final PDF for formatting reasons. These tables will be published as supplementary material.

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We encourage authors to make the figures and visual elements of their articles accessible for the visually impaired. An effective use of color can help people

with low visual acuity, or color blindness, understand all the content of an article.

These guidelines are easy to implement and are in accordance with the W3C Web Content Accessibility Guidelines (WCAG 2.1), the standard for web accessibility best practices.

Ensure sufficient contrast between text and its background
People who have low visual acuity or color blindness could find it difficult to read text with low contrast background color. Try using colors that provide maximum contrast.

WC3 recommends the following contrast ratio levels:

- Level AA, contrast ratio of at least 4.5:1
- Level AAA, contrast ratio of at least 7:1

You can verify the contrast ratio of your palette with these online ratio checkers:

- WebAIM
- Color Safe

Avoid using red or green indicators
More than 99% of color-blind people have a red-green color vision deficiency.

Avoid using only color to communicate information
Elements with complex information like charts and graphs can be hard to read when only color is used to distinguish the data. Try to use other visual aspects to communicate information, such as shape, labels, and size. Incorporating patterns into the shape fills also make differences clearer;

D. Supplementary material

Data that are not of primary importance to the text, or which cannot be included in the article because they are too large or the current format does not permit it (such as videos, raw data traces, and PowerPoint presentations), can be uploaded as supplementary material during the submission procedure and will be displayed along with the published article. All supplementary files are deposited to figshare for permanent storage and receive a DOI.

Supplementary material is not typeset, so please ensure that all information is clearly presented without tracked changes/highlighted text/line numbers, and the appropriate caption is included in the file. To avoid discrepancies between

the published article and the supplementary material, please do not add the title, author list, affiliations or correspondence in the supplementary files.

The supplementary material can be uploaded as:

- data sheet (Word, Excel, CSV, CDX, FASTA, PDF or Zip files)
- presentation (PowerPoint, PDF or Zip files)
- image (CDX, EPS, JPEG, PDF, PNG or TIF/TIFF),
- table (Word, Excel, CSV or PDF)
- audio (MP3, WAV or WMA)
- video (AVI, DIVX, FLV, MOV, MP4, MPEG, MPG or WMV).

Technical requirements for supplementary images:

- 300 DPIs
- RGB color mode.

For supplementary material templates (LaTeX and Word), see our supplementary material templates.

E. References

Frontiers' journals use one of two reference styles, either Harvard (author-date) or Vancouver (numbered). Please check our help center to find the correct style for the journal to which you are submitting.

- All citations in the text, figures, or tables must be in the reference list and vice-versa
- The names of the first six authors followed by et al. and the DOI (when available) should be provided
- Given names of authors should be abbreviated to initials (e.g., Smith, J., Lewis, C.S., etc.)
- The reference list should only include articles that are published or accepted
- Unpublished data, submitted manuscripts, or personal communications should be cited within the text only, for article types that allow such inclusions
- For accepted but unpublished works use 'in press' instead of page numbers
- Data sets that have been deposited to an online repository should be included in the reference list. Include the version and unique identifier when available
- Personal communications should be documented by a letter of permission
- Website URLs should be included as footnotes

- Any inclusion of verbatim text must be contained in quotation marks and clearly reference the original source
- Preprints can be cited as long as a DOI or archive URL is available, and the citation clearly mentions that the contribution is a preprint. If a peer-reviewed journal publication for the same preprint exists, the official journal publication is the preferred source. See the preprints section for each reference style below for more information.

F. Harvard reference style (author-date)

Many Frontiers journals use the Harvard referencing system; to find the correct reference style and resources for the journal you are submitting to, please visit our help center. Reference examples are found below, for more examples of citing other documents and general questions regarding the Harvard reference style, please refer to the Chicago Manual of Style.

a) In-text citations

- For works by a single author, include the surname, followed by the year
- For works by two authors, include both surnames, followed by the year
- For works by more than two authors, include only the surname of the first author followed by et al., followed by the year
- For humanities and social sciences articles, include the page numbers.

b) Reference list examples

Article in a print journal
Sondheimer, N., and Lindquist, S. (2000). Rnq1: an epigenetic modifier of protein function in yeast. Mol. Cell. 5, 163-172.

Article in an online journal
Tahimic, C.G.T., Wang, Y., Bikle, D.D. (2013). Anabolic effects of IGF-1 signaling on the skeleton. Front. Endocrinol. 4:6. doi: 10.3389/fendo.2013.00006

Article or chapter in a book
Sorenson, P. W., and Caprio, J. C. (1998). "Chemoreception," in The Physiology of Fishes, ed. D. H. Evans (Boca Raton, FL: CRC Press), 375-405.

Book
Cowan, W. M., Jessell, T. M., and Zipursky, S. L. (1997). Molecular and Cellular Approaches to Neural Development. New York: Oxford University Press.

Abstract
Hendricks, J., Applebaum, R., and Kunkel, S. (2010). A world apart? Bridging the gap between theory and applied social gerontology. Gerontologist 50, 284-293. Abstract retrieved from Abstracts in Social Gerontology database. (Accession No. 50360869)

Website
World Health Organization. (2018). E. coli. https://www.who.int/news-room/fact-sheets/detail/e-coli [Accessed March 15, 2018].

Patent
Marshall, S. P. (2000). Method and apparatus for eye tracking and monitoring pupil dilation to evaluate cognitive activity. U.S. Patent No 6,090,051. Washington, DC: U.S. Patent and Trademark Office.

Data
Perdiguero P, Venturas M, Cervera MT, Gil L, Collada C. Data from: Massive sequencing of Ulms minor's transcriptome provides new molecular tools for a genus under the constant threat of Dutch elm disease. Dryad Digital Repository. (2015) http://dx.doi.org/10.5061/dryad.ps837

Theses and dissertations
Smith, J. (2008) Post-structuralist discourse relative to phenomological pursuits in the deconstructivist arena. [dissertation/master's thesis]. [Chicago (IL)]: University of Chicago

Preprint
Smith, J. (2008). Title of the document. Preprint repository name [Preprint]. Available at: https://persistent-url (Accessed March 15, 2018).

2. Vancouver reference style (numbered)

Many Frontiers journals use the numbered referencing system; to find the correct reference style and resources for the journal you are submitting to, please visit our help center.

Reference examples are found below, for more examples of citing other documents and general questions regarding the Vancouver reference style, please refer to Citing Medicine.

a) In-text citations

- Please apply the Vancouver system for in-text citations
- In-text citations should be numbered consecutively in order of appearance in the text – identified by Arabic numerals in the parenthesis (use square brackets for physics and mathematics articles).

b) Reference list examples

Article in a print journal
Sondheimer N, Lindquist S. Rnq1: an epigenetic modifier of protein function in yeast. Mol Cell (2000) 5:163-72.

Article in an online journal
Tahimic CGT, Wang Y, Bikle DD. Anabolic effects of IGF-1 signaling on the skeleton. Front Endocrinol (2013) 4:6. doi: 10.3389/fendo.2013.00006

Article or chapter in a book
Sorenson PW, Caprio JC. "Chemoreception". In: Evans DH, editor. The Physiology of Fishes. Boca Raton, FL: CRC Press (1998). p. 375-405.

Book
Cowan WM, Jessell TM, Zipursky SL. Molecular and Cellular Approaches to Neural Development. New York: Oxford University Press (1997). 345 p.

Abstract
Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, editor. Genetic Programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3–5; Kinsdale, Ireland. Berlin: Springer (2002). p. 182–91.

Website
World Health Organization. E. coli (2018). https://www.who.int/news-room/fact-sheets/detail/e-coli [Accessed March 15, 2018].

Patent
Pagedas AC, inventor; Ancel Surgical R&D Inc., assignee. Flexible Endoscopic Grasping and Cutting Device and Positioning Tool Assembly. United States patent US 20020103498 (2002).

Data
Perdiguero P, Venturas M, Cervera MT, Gil L, Collada C. Data from: Massive sequencing of Ulms minor's transcriptome provides new molecular tools for a genus under the constant threat of Dutch elm disease. Dryad Digital Repository. (2015) http://dx.doi.org/10.5061/dryad.ps837

(1) **Theses and dissertations**

Smith, J. (2008) Post-structuralist discourse relative to phenomological pursuits in the deconstructivist arena. [dissertation/master's thesis]. [Chicago (IL)]: University of Chicago

Preprint
Smith, J. Title of the document. Preprint repository name [Preprint] (2008). Available at: https://persistent-url (Accessed March 15, 2018).