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Risk assessment models in genetics clinic for array comparative genomic hybridization: Clinical information can be used to predict the likelihood of an abnormal result in patients

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Abstract. Array comparative genomic hybridization (aCGH) testing can diagnose chromosomal microdeletions and duplications too small to be detected by conventional cytogenetic techniques. We need to consider which patients are more likely to receive a diagnosis from aCGH testing versus patients that have lower likelihood and may benefit from broader genome wide scanning. We retrospectively reviewed charts of a population of 200 patients, 117 boys and 83 girls, who underwent aCGH testing in Genetics Clinic at Rhode Island hospital between 1 January/2008 and 31 December 2010. Data collected included sex, age at initial clinical presentation, aCGH result, history of seizures, autism, dysmorphic features, global developmental delay/intellectual disability, hypotonia and failure to thrive. aCGH analysis revealed abnormal results in 34 (17%) and variants of unknown significance in 24 (12%). Patients with three or more clinical diagnoses had a 25.0% incidence of abnormal aCGH findings, while patients with two or fewer clinical diagnoses had a 12.5% incidence of abnormal aCGH findings. Currently, we provide families with a range of 10–30% of a diagnosis with aCGH testing. With increased clinical complexity, patients have an increased probability of having an abnormal aCGH result. With this, we can provide individualized risk estimates for each patient.

Keywords: aCGH testing, micro-array, medical management, genetic diagnosis, genetic testing

1. Introduction

Clinical geneticists have pioneered the use of risk assessment models in clinical medicine. In the prenatal setting, the quad screen using serum markers identifies pregnancies at increased risk for chromosomal abnormalities. In cancer counseling, risk assessment models are routinely used to identify families at high risk for hereditary breast and ovarian cancer and colon cancer. In pediatric clinics, a risk assessment model is available to help identify which patients are at risk for carrying phosphatase and tensin homolog (PTEN) mutations [http://www.lerner.ccf.org/gmi/ccscore/]. To our knowledge, no risk assessment tools exist for consideration

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of perhaps the most currently utilized test in clinical genetics practice: aCGH testing [1].

The genetics community has written extensively on how aCGH testing is currently considered a first-tier diagnostic test in patients with global developmental delay, intellectual disability, multiple congenital anomalies and autism spectrum disorders [1]. The American College of Medical Genetics recommends aCGH testing for these patients [2]. aCGH has up to a 30% diagnostic yield, as opposed to a karyotype with a 5% yield.

aCGH testing can have an important impact on medical management, and thus has become standard of care. Coulter and Miller performed a retrospective chart review of aCGH testing on 1792 patients with global developmental delay/intellectual disability, autism spectrum disorders, and congenital anomalies and found that aCGH results influenced management in a majority of patients with abnormalities as well as in a significant number of patients with variants of unknown significance [3]. Possible changes in medical management included improved access to services, and referrals to specialists, as well as avoiding unnecessary testing such as a muscle biopsy. Saam et al. [4] surveyed 14 physicians of 48 patients, and found that 71% of patients had management changes following aCGH, including the ability for physicians to provide patients' families with recurrence risks for subsequent pregnancies.

At this point, since aCGH is the obvious first choice in patients with undiagnosed intellectual disability and global developmental delay in cases when clinical history and physical examination do not reveal an obvious etiology, risk assessment models might improve pre-test counseling regarding the probability that a child will have an abnormality. In addition, in the burgeoning field of genetics, we are at a crossroads, about to face the daunting task of incorporating whole genome/exome sequencing or next-generation sequencing into practice. The cost of whole genome/exome sequencing promises to be cheaper than aCGH in the near future [5]. Certainly, the first step will be deciding which patients should be tested with aCGH versus these newer technologies, like exome sequencing, that focus on looking not for copy variants but sequence changes. Risk assessment models for aCGH may ultimately help standardize and direct testing decisions in cases of unexplained developmental disability or delay.

To develop risk assessment models for aCGH, clinical information must be analyzed to see if predictions regarding abnormal versus normal results in patients can be made. To date, only one study has investigated the relationship between certain clinical diagnosis and the likelihood of an abnormal aCGH result. Ezugha et al. [6] analyzed charts from a neurology and genetics clinic. Their collected data on 82 children with normal karyotypes included age, sex, and the presence of what they termed clinical variables intellectual disability, global developmental delay, autism, learning disability, hypotonia, dysmorphic features, and epilepsy. Results showed that patients with ≥4 clinical variables demonstrated a 30.5% incidence of abnormal chromosomal micro-array findings, compared with 8.7% of patients with ≤3 clinical diagnosis [6].

The goal of our study was to investigate whether patients in our genetics clinic with more complex medical histories were more likely to have abnormal results and ultimately predict the future probability of individual patients having a normal versus abnormal result. In addition, we hoped to identify individual risk factors that increase the likelihood of a diagnosis. This research was reviewed and approved by the Rhode Island Hospital Institutional Review Board.

2. Materials and methods

We retrospectively reviewed the charts of 200 pediatric patients, 117 boys (58.5%) and 83 girls (40.5%), referred to Genetics Clinic at Rhode Island Hospital between January 2008 and December 2010 who underwent aCGH testing. In almost all cases, a 180K array was used. Mean age at presentation was 4.04 ± 4.75 yr.

Data collected included sex, age at initial clinical presentation, aCGH result, and six possible clinical diagnosis: history of seizure, autism, dysmorphic features, global developmental delay/intellectual disability, hypotonia, and failure to thrive. For each patient, each clinical diagnosis was coded "1" if it was present and "0" if it was absent. The frequency of clinical diagnoses is presented in appendix 1. We also identified the number of clinical diagnoses for each patient. One hundred and forty two (71%) patients had a normal result, 24 (12%) had a variant of unknown significance (which may or may not be diagnostic) and 34 (17%) had an abnormal and definitively diagnostic result. Deletions comprised 74% of the chromosomal abnormalities, including chromosome 2 (n = 3), 3 (n = 6), 5 (n = 5), 16 (n = 3), 18 (n = 3), 22 (n = 3), and X (n = 3). The abnormal aCGH results are presented in appendix 2. Nineteen mothers and 14 fathers of the 34 children with abnormal micro-array results received genetic testing

in order to determine if the child had a de novo or inherited mutation. Of these, four parents manifested abnormal micro-array results identical to his/her child, indicating that the child had an inherited mutation. This finding implies that in the majority of cases, the abnormal micro-array finding was the result of a de novo mutation. The number of patients who received a genetic diagnosis after a normal aCGH result was 11 out of 142. Of these 11, six went on to second tier testing to another diagnosis and five used the micro-array for a diagnosis of exclusion to support the most likely diagnosis.

In patients with an abnormal micro-array, 38.2% had a family history of delay or autism and 17.6% had a family history of miscarriages or stillbirths. In patients with a variant of unknown significance on micro-array, 45.8% had a family history of delay or autism and 33.3% had a family history of miscarriages or stillbirths. In patients with a normal micro-array, 43.7% had a family history of delay or autism and 26.1% had a family history of miscarriages or stillbirths. Two patients had consanguineous parents.

We compared patients who received an abnormal result on micro-array to patients who received a normal result. We excluded patients with a variant of unknown significance. The analysis was performed in three steps. First, we performed a logistic regression analysis to identify the relationship between the number of clinical diagnosis and the micro-array result (normal or abnormal). Next, we cross-tabulated the number of clinical diagnosis with the micro-array result to refine the model further. Finally, we performed a logistic regression to identify the relationship between micro-array result and the specific clinical diagnosis.

3. Results

3.1. Logistic regression: number of clinical presentations and micro-array result

Each of the patients in our population presented with zero to six clinical diagnoses. We performed a logistic regression using the number of clinical diagnoses as the independent variable and micro-array result (normal = 0, abnormal = 1) as the response variable. The results are presented in table 1.

The value of the coefficient for clinical diagnoses (0.286) indicates that as the number of clinical diagnoses in this population increases, the odds of an abnormal micro-array result also increases. The probability of an abnormal micro-array result can be calculated for any number of clinical diagnoses using the formula: Equation 1.

Table 1
Results of logistic regression of number of clinical diagnoses on micro-array results

Coefficients	Value	Standard error
Intercept	–2.306	0.479
Clinical diagnoses	0.286	0.136

Table 2
Probability and relative risk of an abnormal micro-array result with number of clinical presentations in a pediatric population at the genetics clinic at Rhode Island Hospital

Clinical diagnoses	*P*	RR
0	0.091	
1	0.117	1.286
2	0.150	1.648
3	0.190	2.098
4	0.238	2.615
5	0.295	3.242
6	0.357	3.923

$$P = e^{a+bx}/(1 + e^{a+bx}),^{7}$$

Where P = the probability of an abnormal micro-array result, e is the base of the natural logarithms (2.718…), a = the intercept (-2.306), b = the regression coefficient (0.286) and x = the number of clinical diagnoses (0 to 6). These results are presented in table 2.

Column 1 gives the number of clinical diagnoses, column 2 gives the probability and column 3 gives the increase in risk of an abnormal result compared with the null clinical diagnoses (clinical diagnosis = 0). Thus, for example, the probability of a patient with six clinical diagnoses having an abnormal result is 35.7%; this represents an almost three-fold increase (2.9) in the probability of an abnormal result. The probability and relative risk of an abnormal micro-array result with various clinical diagnoses are presented in table 3.

3.2. Analysis of frequency distributions

To refine the model further, we inspected the frequencies of patients with varying numbers of clinical diagnoses, specifically, patients with two or fewer clinical diagnoses compared with three or more clinical diagnoses. These results are, presented in table 4.

Table 3
Probability and relative risk of an abnormal micro-array result with various clinical diagnoses in a pediatric population at the genetics clinic at Rhode Island Hospital*

Clinical diagnoses	*P*	RR
None	0.086	–
Developmental delay/intellectual disability	0.142	1.651
Developmental delay/intellectual disability + seizure	0.218	2.535
Developmental delay/intellectual disability + seizure + dysmorphic features	0.271	3.151
Developmental delay/intellectual disability + seizure + dysmorphic features + hypotonia	0.360	3.181
Developmental delay/intellectual disability + seizure + dysmorphic features + hypotonia + autism	0.376	4.372
Developmental delay/intellectual disability + seizure + dysmorphic features + hypotonia + autism + failure to thrive	0.390	4.535

*Table 5 shows that the probability of an abnormal micro-array result for patients having only global developmental delay/intellectual disability is 14.2%. This risk, while low, is a 65% increased risk of an abnormal result over that of patients with no clinical diagnoses. On the other hand, the risk of an abnormal micro-array result for a patient with all six clinical diagnoses is 39%, representing a 350% increase in risk over that of patients with no clinical diagnoses. As was the case with the previous regression analysis, the relative risk values indicate that there is a clinically meaningful effect associated with the use of these clinical diagnoses to predict micro-array result.

Table 4
Alternative outcomes of 200 patients who underwent array comparative genomic hybridization analysis and had two clinical diagnoses or less vs. more than two clinical diagnoses

Number of clinical diagnoses	Array comparative genomic hybridization results		
	Normal n (%)	Variant of unknown significance n (%)	Abnormal n (%)
Patients with two clinical diagnoses or less	94 (73.4%)	18 (14.1%)	16 (12.5%)
Patients with three clinical diagnoses or more	48 (66.7%)	6 (8.3%)	18 (25.0%)

Patients with two or fewer clinical diagnoses had a 12.5% incidence of abnormal aCGH findings while patients with three or more clinical diagnoses had a 25.0% incidence of abnormal aCGH findings Thus, patients with three or more clinical diagnoses had a 100% increase in abnormal micro-array results over those with two or fewer. These results are consistent with those of the logistic regression discussed above. No difference in abnormal aCGH results was found upon analysis of age or sex.

3.3. Logistic regression: individual clinical diagnoses and micro-array result

The final step in the analysis was to perform a logistic regression to assess the relationship between the individual clinical diagnosis and micro-array result [7]. The clinical diagnoses chosen for inclusion in the model were global developmental delay and intellectual disability combined (global developmental delay/intellectual disability), failure to thrive, seizure, autism, dysmorphic features, and hypotonia. Each clinical diagnosis was coded "1" if present or "0" if absent. The logistic regression model is presented in table 5. An example of the use of the regression model is presented in appendix 3.

Table 5
Logistic regression of clinical diagnoses on micro-array results

Coefficients	Value	Standard error
Intercept	–2.3637	0.572
Failure to thrive	0.0563	0.580
Developmental delay/ intellectual disability	0.5634	0.552
Autism	0.0718	0.576
Seizure	0.5237	0.449
Dysmorphic features	0.3863	0.425
Hypotonia	0.3132	0.444

The absolute value of the coefficient associated with each clinical diagnosis indicates the relative strength of that clinical diagnosis in contributing to the likelihood of an abnormal micro-array result in this population. Thus, global developmental delay/intellectual disability is the strongest contributor, followed by seizure, dysmorphic features, and hypotonia. Autism diagnosis and failure to thrive do not contribute substantially to the model.

4. Discussion

In our study, aCGH had a diagnostic yield of 17%, similar to the rate previously reported in larger studies, including Miller et al. [1] who observed a diagnostic yield of 15–20% when reviewing 21,698 patients tested by chromosomal micro-array. In our study, the likelihood of an abnormal micro-array result increased with the complexity of patients' medical history and presentation. Patients who received diagnostic results on aCGH were more likely to have two or more dysmorphic features, an additional congenital anomaly, more severe degree of intellectual disability, and more severe growth problems compared to patients that remained undiagnosed. Patients who received a normal result only had only a 7.7% chance of receiving a genetic diagnosis on subsequent genetics follow-up visits where additional studies were considered, which emphasizes the potential for newly emerging technologies to increase our diagnostic abilities.

Currently, we provide families with a range of 10–30% of a diagnosis with aCGH testing. It may be possible to provide more specific risk estimates: for instance for families of patients with one or two major findings, such as only autism or only dysmorphic features, the chance of an abnormality is likely less than 10%, but in patients with three or more diagnoses, the chance is about 20% or greater. In clinical practice, it is sometimes difficult to determine whether to begin with aCGH versus a single gene test, such as in a patient with macrocephaly and global developmental delay, where PTEN testing may be indicated. A clinician might compare likelihood ratios in the PTEN relative risk model versus the predicted chance for aCGH testing before making a decision and tier testing accordingly. In the near future, in those patients that have less likelihood of returning with an answer on aCGH testing, such as a patient with autism and no other features, next generation sequencing or whole genome exome technologies may soon be considered first-tier. In those patients who have normal results on aCGH testing, and after subsequent evaluation do not receive a diagnosis, our results remind us that the next step may be to watch and wait for new technologies to become more standard.

This study was limited in that it is a retrospective design. It will be informative to continue collecting micro-array results and clinical diagnoses from future patients at the genetics clinic to enhance our model's precision. It would also be informative to follow this cohort of patients in a prospective study to determine whether their micro-array results are predictive of future health consequences.

The conclusions of this study are limited by the fact that the study population was drawn from patients referred to a genetics clinic in a large northeast metropolitan area. Our results apply to the population we studied and any attempt to generalize to a wider population must be, undertaken with this in mind. The results could vary with a broader geographic population or with a different population. That said these results are consistent with those of previous smaller studies [6]. Future research may validate our findings and focus on clinical factors that can stratify patients more accurately to predict test results.

Appendix 1
Frequency of clinical diagnoses in 200 patients who underwent array comparative genomic hybridization analysis

Clinical diagnosis	Array comparative genomic hybridization results			
	Normal	Variants of unknown significance	Abnormal	All
Seizure	27	3	10	40
Autism diagnosis	21	4	5	30
Dysmorphic features	84	16	23	123
Developmental delay/intellectual disability	105	16	29	150
Hypotonia	36	4	12	52
Failure to thrive	18	1	5	24
Patients with no clinical diagnosis	7	2	1	10
Patients with one clinical diagnosis	43	6	5	54
Patients with two clinical diagnoses	44	10	10	64
Patients with three clinical diagnoses	33	6	13	52
Patients with four clinical diagnoses	14	0	5	19
Patients with five clinical diagnoses	1	0	0	1

Appendix 2
Abnormal array comparative genomic hybridization results

1	1q21.1 deletion: arr cgh 1q21.1q21.1(143,250,692-147,134,175)x1
2	1q25.3-q32.1 & 15q11.2 deletion: ish del(1)(q25.3q31.1)(RP11-162L13-), del(15)(q11.2q11.2)(RP11-80H14-, RP11-452H4dim).arr 1q25.3q32.1(181,421,767-201,545,623)x1 dn, 15q 11.2 (20,202,654-21,256,269)x1 dn
3	2q13 microdeletion: 2q13(108666764-109784684)x1
4	arr 2p16.3 (50,835,617-51,167,875)
5	microdeletion of 2q37.3: ish del(2)(q37.3q37.3)(RP11-475G3-). arr cgh 2q37.3 (239,403,184-242,951,149)x1 dn
6	deletion of 1 clone of a least 1-2.3kb from the short arm of a chrom 3, gain of at least 50 kb at band 16 P 13.11 from the short arm of chom 16
7	derivative chromosome 3 and 22q deletion: ish der(3)t(3;22)(p?26.3;q11.21)dn,-22(RP11-797C18+,RP11-652F11/RP11-316L10-, RP11-359L2+) .arr 22q11.1q11.21(14,636,331-18,841, 786)x1
8	3p26.3 deletion: arr 3p26.3(2,847,720-2,987,634)x1
9	ish del(3)(p26.3p26.3)(RP11-63O1).arr cgh 3p26.3(2,070,200-2,799,047)x1 mat
10	3q microduplication: nuc ish 3q27.3(RP11 -358N22x3) mat.arr 3q27.3q28(187,586,969-192,122,490)x3
11	3q26.32q29(RP11-253H10-RP11-23M2)x3
12	4.0 Mb Xp28 duplication: arr cghXq28(150324929-154405159)x3
13	5p deletion: 5pter5p15.33(0-4,174,010)x1
14	5p interstitial deletion: ish del(5)(p14.3p14.3)(RP11-645M7-).arr5p15.1p13.3(17,467,677-30,117,148)x1
15	5q deletion: 5q15q21.3 (92,002,474-106,503,702)x1
16	5q deletion: 5q23.1q23.2(120,075,70-126,188,388)x1
17	6p deletion: 6p21.1 (41,411,592-43,199,002)x1pat
18	7q11.23 microdeletion: ish del(7)(q11.23q11.23)(RP11-100C23-). arr 7q11.23(72,360,373-73,860,847)x1, Williams syndrome
19	Characterized by loss at the subtelomeric region of the long arm of chromosome 9
20	11p duplication, arr cgh 11p15.4 (RP11-139I6)x3
21	ish del(12)(q12-q13.11)(RP11-135O4-). nuc ish(RP11-135O4)x1
22	16p deletion: ish del(16)(p11.2p11.2)(RP11-114A14-,RP11-18O13-),16q21(RP11-1072M5x2).arr cgh 1p11.2 (29,410,154-30,181,574)x1
23	disease associated copy # change: 16p13.11 (14,825,694-16,479,429)x1 dn
24	loss 16q24: 16q24.2q24.3(85990715-87234084)x1
25	17p duplication: 17p11.2 (RP11-816G17x3).arr cgh 17p11.2(16698089-20152788)x3
26	chom 18 deletions and dupication: 18p11.32p11.21(0-12,574,952)x1,18p11.21(12,574,952-14,941,330)x3,18q21.32q23 (54,842,973-76,117,153)x1
27	18q deletion: 18q21.31(53165621-76111164)x1
28	18q12.3q21.1 interstitial deletion: arr 18q12.3q21.1(41,465,121-44,032,219)x1
29	20pter-p12.1 duplication: ish der(1)t(1;20)(q44;q12.1)(378I20+;RP11-65G18+).arr20p13p12.1(0-15,604,943)x3
30	20q13.13-20q13.2 duplication: 20q13.13q13.2(48,238,251-50,515,181)x3
31	22q deletion: 22q11.21 (17085801-20131804)x1
32	22q duplication/deletion: 22q13.31q13.33(45,490,295-49,107,688)x3, 22q13.33qter(49,107,688-49,691,432)x1
33	XP11.22 duplication: arr Xp11.22(53,477,391-54,128,888)x2 mat
34	Gain of Xp: Xp22.31(6317139-7841856)x3

Appendix 3
Example of the use of the regression model to predict the likelihood of abnormal micro-array results*

As with the regression model comparing the number of clinical diagnoses versus micro-array result (See Equation 1), it is possible to calculate the probability of an abnormal micro-array result given any clinical diagnosis or combination of clinical diagnoses. In this case the formula used to calculate the probability of an abnormal result is:

Equation 2

$$p = e^{a+b(0)x(0)+\ldots+b(n)x(n)} / \left(1 + e^{a+b(0)x(0)+\ldots+b(n)x(n)}\right), n = 1, 6^{8}$$

*Some selected results are presented in table 5. The results in this table are presented to illustrate the application of the model. Similar results could be calculated for any combination of clinical diagnoses we included in the model. Table 5 gives the probability of an abnormal result and relative risk associated with various combinations of clinical diagnoses and micro-array result.

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