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Prediagnostic biomarkers for early detection of glioma—using case–control studies from cohorts as study approach

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Abstract

Background. Understanding the trajectory and development of disease is important and the knowledge can be used to find novel targets for therapy and new diagnostic tools for early diagnosis.

Methods. Large cohorts from different parts of the world are unique assets for research as they have systematically collected plasma and DNA over long-time periods in healthy individuals, sometimes even with repeated samples. Over time, the population in the cohort are diagnosed with many different diseases, including brain tumors.

Results. Recent studies have detected genetic variants that are associated with increased risk of glioblastoma and lower grade gliomas specifically. The impact for genetic markers to predict disease in a healthy population has been deemed low, and a relevant question is if the genetic variants for glioma are associated with risk of disease or partly consist of genes associated to survival. Both metabolite and protein spectra are currently being explored for early detection of cancer.

Conclusions. We here present a focused review of studies of genetic variants, metabolomics, and proteomics studied in prediagnostic glioma samples and discuss their potential in early diagnostics.

Keywords

genetic variants | glioblastoma | metabolites | prediagnositic sample | proteins.

Several reviews have highlighted that a liquid biopsy in the form of a blood sample could potentially be useful in glioma diagnostics either by screening of high-risk individuals or at certain symptoms.[1–3] The development of cancer has been suggested to start 7–8 years before diagnosis in breast and colorectal cancer, and recent studies have suggested a similar trajectory for glioma, which provides a strong rationale to study biomarkers that can be used for detection of these diseases at an earlier stage.[4,5] Worldwide there are several cohorts with individuals that have given blood samples while healthy. These cohorts are followed over time and linkage to diagnostic registries allows the identification of individuals that later develop glioma, providing a unique opportunity to investigate the potential role of biomarkers for early detection of glioma. Studies nested within the cohorts are efficient, especially for investigating the useful biomarkers. Controls can be randomly chosen from the cohort who have not been diagnosed at the same time point as the corresponding case, that is, nested case–control study design. In addition to matching on the time of diagnosis, controls can also be matched on several factors such as age at blood sampling, gender, and time point of blood sampling to reduce confounding. An alternative study design, case–cohort design, is using a case set for different diseases and comparing with cohort controls, that is, a subcohort that represents the whole cohort.[6]

The current review highlights some of the existing studies performed in prediagnostic samples with different omics techniques.

Table 1. Summary of included studies

Year	Author	Country	Study design	Sample size	Sample collection time prior to diagnosis	Biomarkers	Measurement	Findings
Genetic predisposition								
2012	Rajaraman et al.	World-wide	Meta-analysis (14 cohorts, 3 case–control studies, and 1 population-based case–only study)	556 cases from cohort studies and 3649 controls		Genome-wide variants	Illumina 550K, 660W, or 610Q	The consistent findings were shown in cohort and case–control study designs. Stronger associations were found for loci rs6010620 (20q,13.33; *RTEL*) and rs2736100 (5p15.33, *TERT*) in cohort studies.
2015	Wibom et al.	Norway	Nested case–control study	598 cases and 595 matched controls	Median: 14.7 y, range: 0.2–35.1 y	11 single nucleotide polymorphisms	Amplified 6-FAM-labeled PCR	The study confirmed the genetic variants within 5 genomic regions: 8q24.21 (CCDC26), 9p21.3 (CDKN2B-AS1), 11q23.3 (PHLDB1), 17p13.1 (TP53), and 20q13.33 (RTEL1).
Metabolites								
2016	Björkblom et al.	Norway	Nested case–control study	110 cases and 110 matched controls	Mean: 12.6 y, SD: 5.1 y	180 known metabolites	GCxGC-TOFMS	9 metabolites (γ-tocopherol, α-tocopherol, erythritol, erythronic acid, myo-inositol, cystine, 2-keto-L-gluconic acid, hypoxanthine and xanthine) were involved in antioxidant metabolism.
2017	Huang et al.	Finland	Nested case–control study	64 cases and 64 matched controls	Median: 9.0 y, interdecile range: 3–20 y	750 known molecules	LC/MS–MS	43 metabolites were associated with glioma before multiple adjustment. 2-Oxoarginine, cysteine, alpha-ketoglutarate, chenodeoxycholate and argininate were inversely associated with overall glioma risk. 7 xanthine metabolites related to caffeine metabolism were higher in cases than in controls. Ascorbate/aldarate and steroid hormone metabolites associated with high-grade glioma.
2020	Jonsson et al.	Sweden	Nested case–control study	132 cases and 132 matched controls	Mean: 11.7 y, SD: 5.6 (baseline); mean 4.2 y, SD: 3 y (repeated); mean: 8 y, SD: 5.4 y (single)	142 metabolites	GC–MS	15 significantly metabolites associated with glioma progression were identified. Higher plasma levels of myo-inositol, cysteine, *N*-acetylglucosamine, creatinine, glycine, proline, erythronic-, 4-hydroxyphenylacetic-, uric-, and aceturic acid were observed in glioma cases.
Proteins								
2007	Lönn et al.	Finland	Nested case–control study	22 cases and 400 unmatched controls	More than 5 y	IGF-I and IGFBP-3	ELISA	IGF-I was inversely associated with glioma but not IGFBP-3.
2011	Schlehofer et al.	EPIC	Nested case–control study	275 cases and 528 matched controls	Median: 8.24 y, range: 29–4981 days	Specific IgE	ImmunoCAP-specific IgE test	Allergic sensitization was inversely associated with glioma, especially pronounced in women.

Table 1. Continued

Year	Author	Country	Study design	Sample size	Sample collection time prior to diagnosis	Biomarkers	Measurement	Findings
2011	Calboli et al.	USA	Nested case–control study	169 cases and 520 matched controls		Total IgE, food, and respiratory allergen-specific IgE	UniCAP and ImmunoCAP fluorescent assays	Borderline elevated total IgE levels (25–100 kU/L) were inversely associated with glioma but not elevated IgE (>100 kU/L). Food allergen-specific and respiratory allergen-specific IgE levels showed no association with glioma.
2011	Rohrmann et al.	EPIC	Nested case–control study	282 cases and 561 matched controls	Median: 4.5 y	IGF-I and IGFBP-3	ELISA	Higher levels of IGF-I were positively associated with low-grade glioma risk and pronounced after adjustment for IGFBP-3, but not with glioma overall.
2012	Schwartzbaum et al.	Norway	Nested case–control study	594 cases and 1177 matched controls	Median: 15 y, interquartile range: 9–20 y	Total IgE and respiratory allergen-specific IgE	ImmunoCAP fluorescent assays	Elevated total IgE (>100 kU/L) levels were inversely associated with glioma risk and found even at least 20 years before diagnosis. Elevated allergen-specific IgE (>0.35 kU(A)/L) levels were inversely associated with glioblastoma in women but not in men.
2015	Ma et al.		Meta-analysis (5 studies in glioma)	2461 cases and 3934 controls (2021 glioma cases and 3446 controls)				Elevated total IgE levels were inversely associated with glioma risk.
2015	Schwartzbaum et al.	Norway	Nested case–control study	487 cases and 487 matched controls	Median: 16 y, interquartile range: 10–22 y	IL4, IL13, IL5, IL6, IL10, IFNG, TGFB2, sIL4RA, sIL13RA2, FOXP3, STAT3, and STAT6	RayBio Human Cytokine Antibody Array Kits	IL4 and sIL4RA were inversely associated with glioma and glioblastoma. TGFB2 was inversely associated with glioblastoma.
2016	Späth et al.	Norway	Nested case–control study	593 cases and 590 matched controls	Mean: 15.2 y, SD: 8.6 y	EGFR and ErbB2	Multiplex immunoassay (Meso Scale Discovery [MSD])	EGFR and ErbB2 levels were associated with glioblastoma risk. High serum ErbB2 concentration was also associated with glioma risk overall ($P = .049$; OR = 1.39, 95% CI = 1.00–1.93).
2017	Schwartzbaum et al.	Norway	Nested case–control study	487 cases and 487 matched controls	Median: 15 y, interquartile range: 9–21 y	277 cytokines	RayBio Human Cytokine Antibody Array Kits	sIL10RB, VEGF, IL4, and sIL4RA were associated with glioma risk. Interaction between IL4 and sIL4RA was existed.sIL10RB, VEGF, beta-Catenin and CCL22 were associated with glioma risk more than 10 years before diagnosis. LIF was inversely associated with glioma within 5 years before diagnosis.

Table 1. Continued

Year	Author	Country	Study design	Sample size	Sample collection time prior to diagnosis	Biomarkers	Measurement	Findings
2018	Brenner et al.	USA	Nested case–control study	457 cases and 457 matched controls	Median: 15 y, interquartile range: 9–21 y	14 cytokines	Four sensitive custom V-PLEX Meso Scale Discovery kits (MSD) and 1 standard MSD kits	IL-15 and IL-16 were inversely associated with glioma risks.
2021	Cote et al.	UK	Cohort	428 537 participants and 417 incident gliomas	Median: 3.8 y	CRP, WBC, NLR, and IGF-I	Beckman Coulter AU5800 analytical platform, LH750 instruments, and DiaSorin Liaison XL analytic platform	IGF-I was associated with glioma risk in women but not men.
2022	Wu et al.	Sweden	Nested case–control study	133 cases and 133 matched controls	Mean: 11.8 y, SD: 5.6 y (baseline); mean: 4.3 y, SD: 3.0 y (repeated); mean: 8 y, SD: 5.4 y (single)	19 proteins	Luminex bead-based commercial assay panels and ELISA assays	sVEGFR2, sTNFR2, sIL-2Rα, and sIL-6R were associated with glioma risk.

Methods

Search Strategy and Study Selection

We performed a comprehensive literature search in PubMed database to identify relevant studies published through February 23, 2022. The searches typically included 4 key terms "glioma," "biomarkers," "early-detection," and "pre-diagnostic sample." We especially focus on the early-detection role of biomarkers. The references of the identified articles were also searched for other relevant articles. Studies included into this review have to meet the following inclusion criteria: (1) Studies used prediagnostic samples to investigate the relationship between biomarkers, including genetic variants, metabolites, and proteins, and glioma risk; (2) Studies in cohort design or nested within cohort design (ie, nested case–control study and case–cohort study). We excluded the studies that were not written in English, not conducted on humans and published as letters, case reports, and meeting records.

Results

Table 1 summarizes the studies that were included in this review. The following section was divided into (1) genetic predisposition, (2) metabolites, and (3) proteins.

Genetic Predisposition

Glioma predisposition caused by highly penetrant gene mutations occurs at low incidence.[7] In addition to these rare mutations, common genetic variants have been linked to the risk of developing glioma.[8–11] The genetic variants may be deeply involved in the biological development of glioma, but the functional mechanisms are only known in a few cases.[12] The fact that the genetic variants in most cases are located in or in close proximity to genes commonly somatically mutated in glioma suggests a functional importance (Figure 1).

The common genetic risk variants for glioma have been confirmed in several independent studies, of which most are using a case–control design. Since an individual's germline genetic variants will not change during the course of disease, causation bias is not an issue in these studies. Selection bias and survival bias are however of more concern in case–control studies of a disease with high disease-related mortality such as glioma. Considering the potential survival bias, Rajaraman et al. compared the findings from 7 susceptibility regions, including TERT, EGFR, CCDC26, CDKN2B, PHLDB1, and RETL1, between cohort and case–control design.[13] The consistent findings were shown in both designs. However, greater association was found in 2 variants (rs6010620 in RETL and rs2736100 in TERT) in cohort studies, which implied a certain extent of survival bias. Wibom et al. demonstrated the potential of using the nested case–control study design to validate the previous GWAS findings.[14] Most genetic variants have an association in the same direction as previous studies, even if there is limited power for significant results.

Genetic variants with small effects can be combined and used as polygenetic risk score to estimate the glioma risk. However, due to low absolute lifetime risk, using genetic variants as biomarkers for early detection in the population-level screening is not useful.[15] Although the common genetic variants alone are not useful for risk prediction in the general population, the genetic variants have been associated with

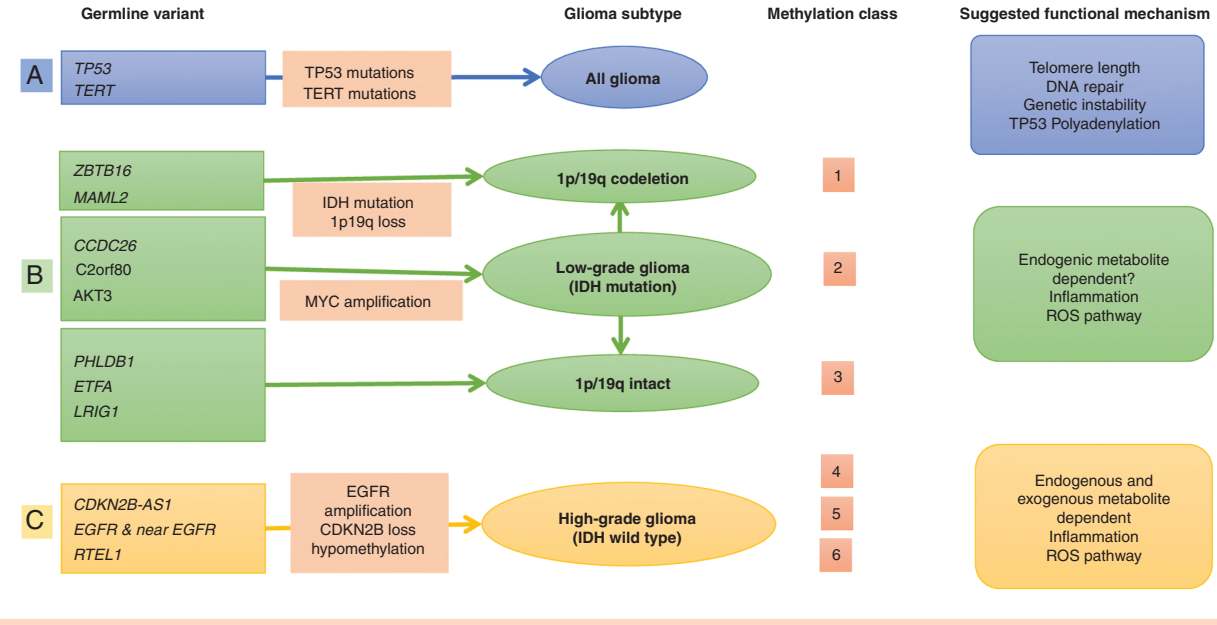

Figure 1. Overview of genomic and metabolomic classification of glioma with hypothesized pathways of importance. The differentiation of glioma subgroups by genomics and metabolomics provides a potential avenue for early diagnoses of glioma by a combined biomarker risk score.

different subgroups of glioma,[16] lending some support that the variants could be included into future combined biomarker panels including other more predictive biomolecules.

Metabolites

Glioma can be defined as a metabolic driven disease both by in 1 subgroup detection of IDH mutations and the metabolic reprogramming of different subgroups.[17] Prediagnostic samples are preferred as the samples taken at glioma diagnoses and at surgery might be affected by several factors that might change, such as stress, treatment with corticosteroids, seizure control drugs, or the sample been taken with arterial or venous puncture.[18] A standard protocol such as sample collection, storage, preparation steps, and operating processes is therefore crucial to control the noise and variation in the experiments for reproducibility. Data preprocessing procedures, that is, identifying metabolites from raw data, and suitable multivariate analysis, also play important roles to find the true association.[19] Few studies have investigated broader metabolite spectra in gliomas using prediagnostic samples. In a study of prediagnostic serum samples from JANUS biobank in Norway, we observed increased levels of several metabolites such as tocopherol, erythritol, and myo-inositol in samples from individuals that later in life have developed glioblastoma.[20] In this study, most of the cases had been sampled 5–20 years before diagnosis. A small study of 64 cases and 64 matched controls that covered wider spectra of metabolites identified 43 associated metabolites, including arginine/proline, antioxidant, and coffee-related metabolites.[21] In another study, 64 glioma cases from Northern Sweden Health and Disease study with repeated plasma samples and 68 single time point cases were analyzed. Tightly matched controls were used from the same sampling year, similar thawing cycles, smoking, and body mass index to be able to separate differences clearly associated with disease. Fifteen significant metabolites associated with the glioma progression were identified by comparing the 2 repeated samples between cases and matched controls. A elevated metabolic pattern in glioma cases was observed in blood plasma of several metabolites for example myo-inositol, cysteine, *N*-acetylglucosamine, creatinine, glycine, and proline.[22] This study highlighted the benefits of using repeated samples for progression pattern analysis. We combined the significant metabolites identified from 3 studies to investigate the potential metabolic pathways (Figure 2).The results suggest

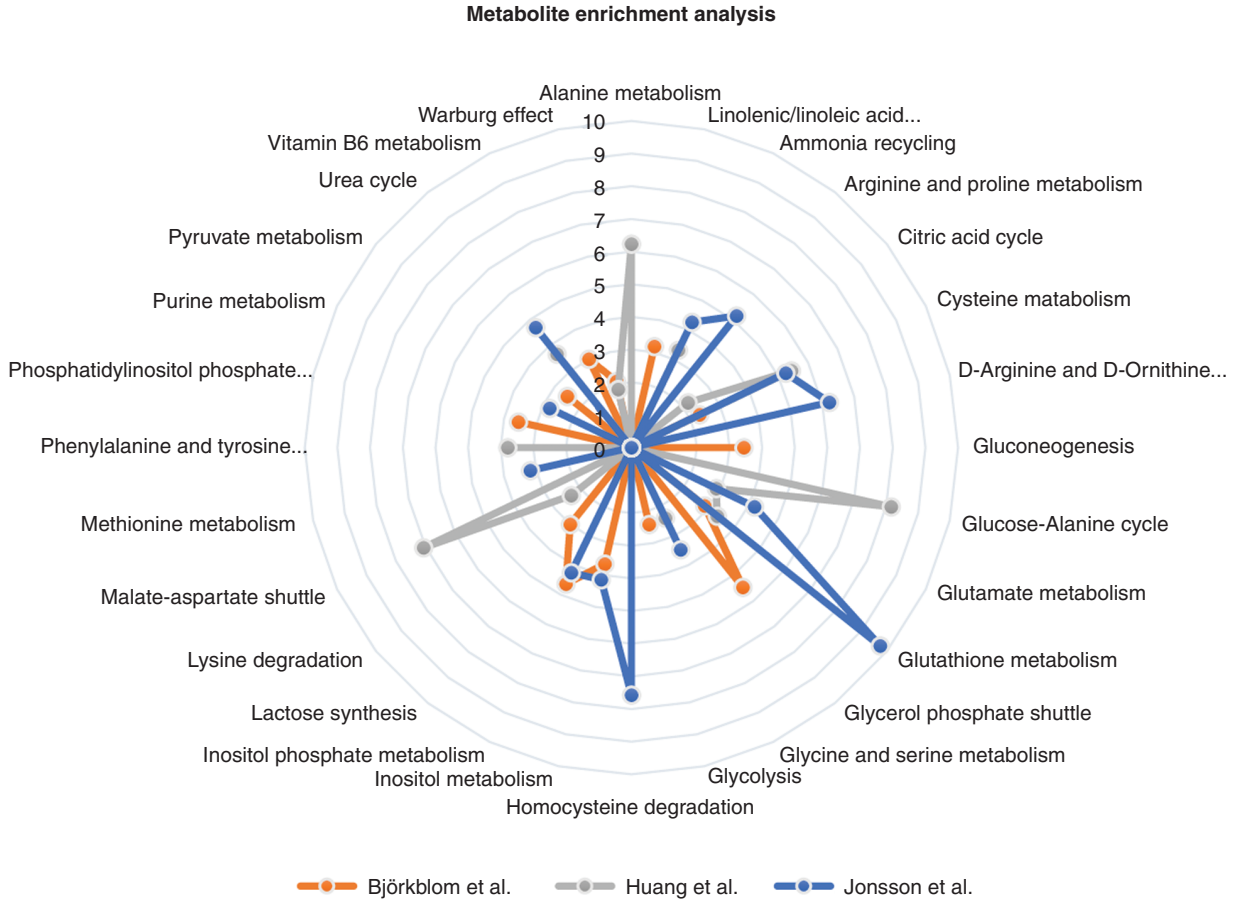

Figure 2. Over representation analysis of significant metabolites found in prediagnostic glioma blood samples enriched in metabolic pathways of the Small Molecule Pathway Database (SMPDB).

that glutathione metabolism, cysteine metabolism, urea cycle, inositol phosphate metabolism, ammonia recycling, glutamate metabolism, inositol metabolism, and glycine and serine metabolism might involve in glioma tumorigenesis. A common limitation of using collected sample from retrospective cohort is that participants often have been recruited 10–20 years ago and some of glioma cases might not able to use new classification for example for IDH mutation status. Larger validation studies of the most promising metabolites are necessary to understand the potential of metabolic biomarkers in different diagnostic settings.

Proteins

Few studies have explored proteomics in prediagnostic samples from glioma cases, likely due to the fact that the techniques still are being developed to be able to study the full proteome. A suggested theory for glioma development is that the innate immune system is of importance of glioma development. The innate immune system could release proteins before the development of symptoms that would render a radiological examination. One potential biological mechanism would be chronical inflammation as a mediator in tumor development. For example, release of inflammatory proteins such as cytokines that have been suggested also for several other cancer categories.[23] In glioma, studies which investigated few proteins have also been done such as immunoglobulin E (IgE), insulin-like growth factor (IGF), EGFR, and ErbB2. IgE and specific IgE are allergy biomarkers which have been used in medical atopy diagnostics. There were 3 nested case–control studies that using prediagnostic samples to investigate the association between serum IgE level and glioma risk.[24–26] Two of them showed significantly negative association, especially in women. A review study concluded negative association between total IgE level but not respiratory allergen-specific IgE.[27] IGF plays important role in human growth and normal brain development and it was associated with increased risk of several cancers.[28] Two nested case–control studies and 1 cohort have investigated the association between IGF-I and IGF-binding proteins and glioma risk in prediagnostic samples.[29–31] Higher circulating IGF-I seemed to increase the risk of low-grade gliomas but reverse causation bias could not be excluded. Interestingly, a Mendelian randomization study using genetic instruments for serum IgE and plasma IGF-I levels could not support their etiological roles of glioma.[32] Späth et al. evaluated the soluble EGFR, a growth factor commonly amplified and mutated in glioblastoma, and ErbB2 concentration in 593 cases and 590 matched controls in JANUS biobank and showed increased levels long before glioma development.[33] Brenner et al. evaluated 14 serum proteins in 457 case–control sets from a military cohort and found that IL-15 and IL-16 were associated with lower glioma risks.[34] In a study of 277 prediagnostic cytokines in the JANUS biobank an observed pattern of 12 cytokines that were stronger with a longer latency before diagnoses, but they were not evident within 5 years of diagnosis.[35] In this study, the levels of 4 proteins were significantly different in glioma cases compared to controls, including sIL10RB, VEGF, IL4, and sIL4RA. In the small sample set of 55 cases that had sampling within 5 years of diagnoses only LIF (leukemia inhibitor factor) and interleukin class protein were significant. In a recent study from our group, we performed a targeted analysis of 19 proteins in repeat samples showing associations of other inflammatory proteins sVEGFR2, sTNFR2, sIL-2Rα, and sIL-6R in glioma patients and matched controls.[36]

Overall, the findings in the proteomic studies of prediagnostic samples so far are still limited due to small sample sizes and the number of proteins. Generally, there is little consistency between studies. A large-scale discovery analysis of protein expression with a broader platform is needed to find relevant candidates that may be validated in independent samples sets. As metabolites, measurement of proteins at the tumor diagnosis might be affected by the disease but an advantage of investigating proteins is that they are less dependent on fasting status than metabolites. However, the limitations are that some proteins might be difficult to detect in the samples that have long freezing periods. Along with the rest of the omics field, larger studies in proteomics with robust validation are warranted.

Conclusions

The development of broad omics analyses with need of limited amount of DNA and plasma have paved the way for good opportunities to explore both the etiology and early detection of disease in the era of personalized medicine and precision diagnostics. Genetic variants have been discovered that gives us an understanding of the etiology of glioma but have limited contribution to risk prediction. Metabolite studies show some promising results but needs further confirmation. Most importantly, the samples need to go along with adequate health data information of the cohort individuals, and information on all preanalytical sample conditions that are corner stones for being able to detect true biological relevant biomarkers. As glioma is a rare disease, collaborative efforts with several independent validations are necessary to find robust biomarkers that can be taken forward to clinical trials.

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